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Treatability Studies of Pesticide Manufacturing Wastewaters Ethylenebisdithiocarbamate Fungicides

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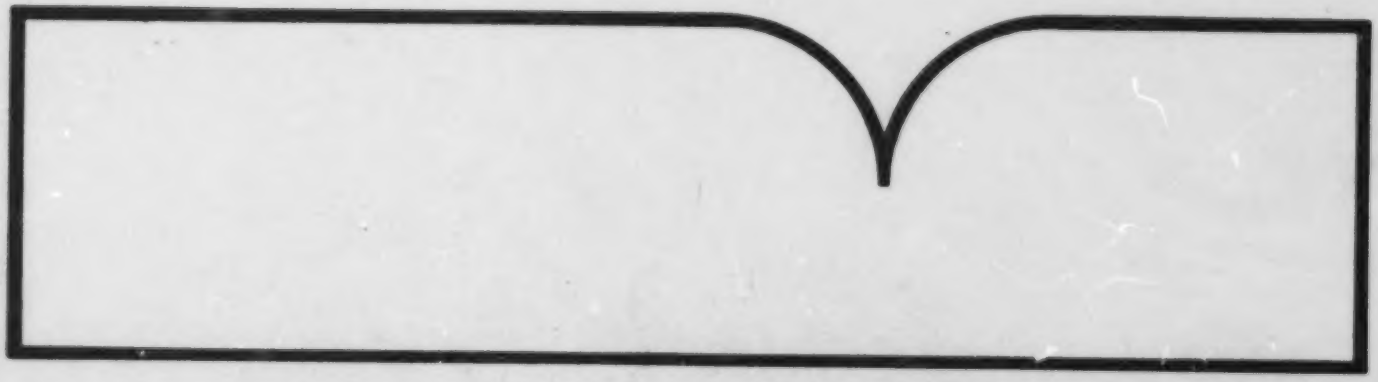

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16. ABSTRACT

The report gives results of laboratory and pilot studies on the biological treatability of wastewaters from the manufacture of ethylenebisdithiocarbamate (EBDC) fungicide. At concentration levels representative of EBDC production units and total plant wastewaters discharged to publicly owned treatment works (POTWs) (1 mg/L), results indicate little reduction in EBDC levels or in ethylene thiourea (ETU), an important decomposition product of EBDC as a result of biological treatment. Results of algal (Selenastrum capricornutum) and fish (Daphnia magna) bioassays on the treated wastewater indicated inhibition of algal growth and fish mobility, although little toxicity was indicated. Chemical oxygen demand (COD) removal by the biological (activated sludge) treatment unit was unaffected by the fungicide wastewater, although nitrification was reduced or absent in these units.

17. KEY WORDS AND DOCUMENT ANALYSIS

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Pesticides	Stationary Sources	06F 06T
Fungicides	Ethylenebisdithiocarbamate	13H
Toxicity		06P
Industrial Processes	Ethylene Thiourea	
Waste Water	Maneb	07C
Oxygen Demand	Chemical Oxygen Demand	07B
Waste Treatment		
Thiocarbamates		
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ETHYLENEBISDITHIOCARBAMATE FUNGICIDES

by

Ruth A. Zweidinger
Rosemary Batten
R. Wayne Hendren
Linda W. Little
Dora Liverman
Mary Warner
Thomas J. Wolff

Research Triangle Institute
Post Office Box 12194
Research Triangle Park, North Carolina 27709

Contract No. 68-02-2688
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Project Officer

David C. Sanchez
Office of Environmental Engineering
and Technology
U. S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

Prepared for
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OFFICE OF RESEARCH AND DEVELOPMENT
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ABSTRACT

Laboratory and comparative pilot studies were conducted on the treatability of wastewaters generated from the manufacture of an ethylenebisdithiocarbamate pesticide. The two wastewaters were (1) the wastewater from the ethylenebisdithiocarbamate (EBDC) production unit and (2) the total plant wastewater. Wastewaters were characterized for pesticide and breakdown products by gas chromatography and high pressure liquid chromatography (HPLC) and for routine wastewater parameters. Toxicity determinations were conducted with Selenastrum capricornutum and Daphnia magna; evaluations were made of pesticide manufacturing wastewaters and of influent and effluent of the pilot activated sludge treatment units. Biological treatability was evaluated in terms of the ability of activated sludge systems to (1) successfully operate on a mixture of municipal wastewater and pesticide wastewater and (2) remove the pesticide and other toxic materials.

Results of this study indicate that the two wastewaters did not affect COD removal in activated sludge units fed dilutions representative of actual treatment conditions. However, nitrification was affected even at these dilutions. Nitrification was severely inhibited in activated sludge units inoculated with sludge from the municipal treatment plant (POTW) which receives the pesticide wastewaters. Nitrification was inhibited to a lesser extent in units inoculated with sludge obtained from the Hope Valley, Durham, N. C., POTW. Removal of ethylenethiourea (ETU), a breakdown product of EBDC, was observed only where nitrification was also observed.

Acute static bioassays performed on wastewater dilutions representative of actual conditions at the POTW indicated little, if any, effect on Daphnia either before or after biological treatment. Algal assays on effluents from biogical treatment units showed that effluents from units fed a mixture of EBDC wastewater and municipal wastewater were no more inhibitory than effluents

from control units receiving municipal wastewater alone. Undiluted effluents from activated sludge units fed total plant wastewater diluted in municipal wastewater strongly inhibited algal growth; however, after dilution to about one-half the original strength, these effluents were no more inhibitory than control effluents. Overall, therefore, these effluents would be expected to have little acute impact on algae and Daphnia when discharged into a receiving stream.

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SECTION 1

INTRODUCTION

In February 1979 Research Triangle Institute (RTI) was requested by the Industrial Environmental Research Laboratory, Research Triangle Park (IERL-RTP) of the U.S. Environmental Protection Agency (EPA) to conduct laboratory and pilot studies of the treatability of a number of pesticide manufacturing wastewaters. The project was designed to investigate the suitability of individual pesticide manufacturing wastewaters for discharge to biological treatment systems, whether publicly owned treatment works (POTW) or onsite systems.

The approach taken with each pesticide manufacturing wastewater is hierarchical in nature, that is, less costly, more available methods of treatment are investigated first. If the pesticide wastewater is judged suitable for biological treatment based on chemical and toxicological evaluation of the wastewater before and after treatment, additional options are not investigated.

If pesticide manufacturing wastewater disrupts biological treatment systems, the possibility of pretreating the waste prior to biological treatment is investigated. Pretreatment options may include pH adjustments, filtration, flocculation, oxidation and others depending on the nature of the wastewater and its chemical composition.

If pretreatment does not improve the performance of activated sludge systems, adsorption techniques may be investigated. These may involve both carbon and resin systems. Physical-chemical treatability of wastewaters is again evaluated as with the biological treatment system (i.e., removal of toxicity and of constituents of interest).

This report describes a study of the treatability of wastewaters resulting from the manufacture of ethylenebisdithiocarbamate (EBDC) fungicides which may include maneb, zineb, mancozeb or metiram.

In a previous study of wastewater from this facility (Little et al., 1980), the wastewater from the final production step of the EBDC type fungicide was employed. The current study was addressed to the biological treatability of (1) the entire wastewater from the EBDC production unit and (2) the total plant effluent, at dilutions representative of the wastewater concentrations entering the POTW to which they are discharged. Sludge obtained from this POTW was used as inoculum in some of the experiments in order to provide organisms acclimated to the wastewaters. Finally, particular attention was directed toward effects of these wastewaters on nitrification and to the ability of biological treatment to remove the fungicide and its breakdown product, ETU, from the wastewater. As with any bench scale study, verification of specific findings should be verified in the field.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

1. At levels representative of their concentration in the influent to the POTW, neither the wastewater from the EBDC production unit nor the total plant wastewater affected the ability of pilot activated sludge units to remove COD.
2. At these levels, the presence of both wastewaters strongly inhibited nitrification in activated sludge units, as compared to control units fed typical domestic wastewater alone.
3. Biological treatment decreased, but failed to eliminate, the amount of ETU in the wastewaters.
4. Nitrification and ETU removal were superior in activated sludge units inoculated with sludge from a plant treating domestic wastewater compared to units inoculated with sludge from the POTW receiving the pesticide wastewater. Nitrifying bacteria are known to be inhibited by thioureas. These findings possibly indicate that continued exposure of sludge to the EBDC wastewaters can reduce the bacterial populations responsible for nitrification and for ETU removal.
5. Results of algal and Daphnia assays in the two wastewaters indicated inhibition of algal growth and Daphnia mobility. Metals analysis indicated this toxicity could be due in part to the presence of Mn and Zn. If so, removal of these metals might be investigated as a means of relieving the toxicity of the wastewaters to aquatic organisms. It should be noted, however, that at dilutions representative of actual conditions at the receiving POTW neither wastewater exhibited much toxicity to Daphnia and algae.
6. Future studies should be conducted to define the effects of ETU and EBDC fungicides on nitrification, since this process, either in a POTW or in a receiving stream, is critical to maintenance of the nitrogen cycle and should be studied further.

7. The public health significance of ETU in the activated sludge (AS) effluents should be investigated.

SECTION 3

SELECTED LITERATURE REVIEW

GENERAL CHEMICAL INFORMATION

Chemical Name

Ethylenebisdithicarbamate type fungicides

CAS Number

012427382-Maneb
012122677-Zineb

Synonyms and Trade Names

Maneb; Carmazine™; Dithane™ M-45; Farmantz™; Fore™; Moromate™

Structure

Ethylenebisdithiocarbamate type fungicides are salts of manganese, zinc, iron or a mixture of salts. The different formulations vary considerably and may be toxicologically distinct from one another. The general formula is

$$[S\text{-}SC\text{-}NH\text{-}CH_2CH_2\text{-}NH\text{-}CS\text{-}S]_xM_y$$

where M is a transition metal or mixture of transition metals.

Chemical Category

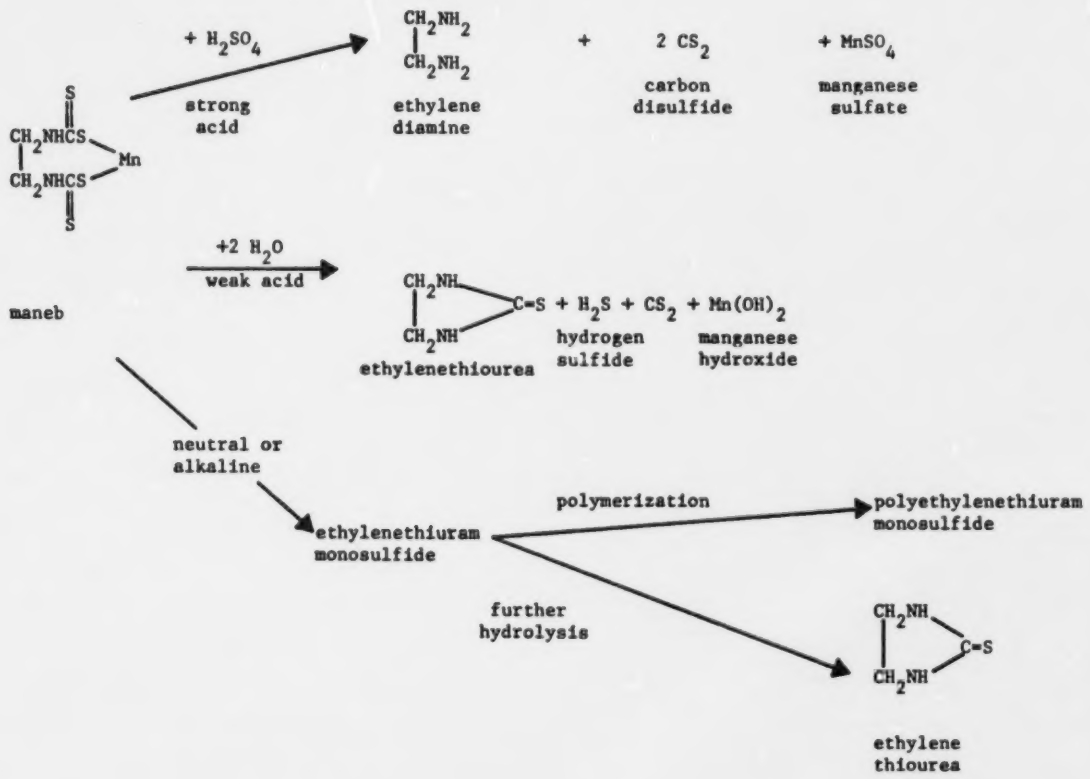
Ethylenebisdithiocarbamate (EBDC)--Others in this class include maneb, mancozeb, zineb, and metiram. Ethylenebisdithiocarbamates are also widely used as slimicides in the paper industry and are used in rubber processing.

Chemical Properties

The properties of the EBDC fungicides can best be characterized by maneb, the manganous salt, with a formula weight of 265. It decomposes before melting. Yellow crystalline solid. Stable to storage in dry air but decomposes in the presence of moisture to give carbon disulfide (CS_2) and ethylene thiourea (ETU). Maneb is insoluble in organic solvents. The apparent aqueous solubility of maneb determined in a previous study was 40 mg/l. Apparent EBDC concentration is defined in terms of CS_2 generated by the sample under the conditions of EBDC analysis (Appendix F). The compounds

determined included EBDC and other compounds generating CS_2 (e.g., ethylene thiuram disulfide or monosulfide).

Hydrolysis reactions of maneb are complex and may occur under acid, alkaline, or neutral conditions, according to Shih and Dal Porto (1975):



Shih and Dal Porto (1975) also note that, in addition to the above reactions, under neutral or alkaline conditions formation of ethylene diisothiocyanate and ethylene diamine may occur. CS_2 evolution accompanies both alkaline hydrolysis and hydrolysis under strong acid conditions. Under some conditions chemical hydrolysis may produce ETU, as indicated in the diagram above. Chemical oxidation may likewise produce ethylenethiuram monosulfide which hydrolyzes to ETU. For these reasons, these authors recommend disposal of commercial maneb preparations by burial or incineration, rather than by chemical treatment. However, for the more dilute pesticide manufacturing wastewaters, one cannot rule out the possible usefulness of hydrolysis under controlled conditions.

ETU is water-soluble [2 g/100 mL at 30°C (Merck Index, 1976)] and stable to sunlight, but the presence of added photosensitizers (such as acetone or riboflavin) causes rapid photolysis; agricultural field waters taken from locations of dithiocarbamate application catalyzed ETU photodecomposition (Crosby, 1976).

Intended Use

Maneb type fungicides are used for control of foliar fungal blights. It is recommended for prevention of early and late blight on tomatoes and potatoes, and can be combined with other fungicides for persistent fungal strains. Ethylenebisdithiocarbamate (EBDC) fungicides are used to control over 400 fungal diseases for protection of over 70 crops.

Mode of Action

Unknown.

Manufacturing Information

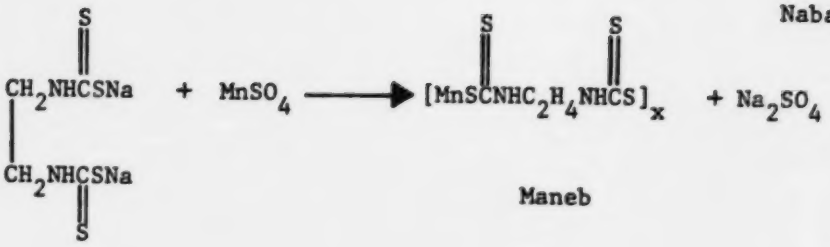
Production of dithiocarbamic acid salts for use as fungicides was estimated in 1974 to be 16.1 x 10^3 metric tons annually; of these 5.4 x 10^3 metric tons were maneb. (Archer et al., 1978)

Manufacturers	Locations
DuPont	LaPorte, TX
Rohm & Haas	Philadelphia, PA

Manufacturing Process

The processes described here are generalized and are not necessarily identical to those in operation at the plant sampled. According to the National Research Council (NRC, 1977) dithiocarbamates can be synthesized by reacting a suitable amine, such as dimethylamine or ethylenediamine, with CS_2 under alkaline conditions to yield the alkaline salt of the alkyl dithiocarbamic acid. These water-soluble salts can then be reacted with aqueous solutions of salts of zinc, manganese, and iron to form precipitates of the corresponding highly insoluble metallosalts. Alternatively, the metallosalts can be produced by reacting metal oxides with the appropriate amine and CS_2. Sittig (1977) describes the reaction chemistry as follows:

Nabam



Maneb

Nabam

Alternatively, EBDC salts can be produced by reacting ethylenediamine with sodium hydroxide solutions and CS_2 to form the disodium ethylenebisdithiocarbamate, which is then reacted with manganous and zinc chloride to form the fungicide (Sittig, 1977). The major raw waste load from the manufacturing process is the wastewater remaining after the precipitate is removed.

As summarized by Sittig (1977), air, liquid, and solid waste problems may occur. The liquid waste stream contains about 1 Kg (2 lb) of salts for each 6 Kg (13 lb) of EBDC salt produced, these salts being primarily sodium sulfate with some manganese sulfate.

HEALTH AND ECOLOGICAL EFFECTS BASED UPON STUDIES OF MANEB

Mammalian Toxicity

Toxicity is rated as low:

Rat, oral LD_{50}: 6750 mg/kg (Fairchild, 1977)

Rat, inhalation, LC_{50}: 3000 mg/kg (Fairchild, 1977)

Recommended allowable daily intake, human: 0.005 mg/kg/day (NRC, 1977)

In animal metabolism studies with ^{14}C-maneb, about 55% was excreted as the metabolites ethylenediamine, ethylenebisthiuram monosulfide (ETM) and ETU, these metabolites associated mainly with the feces; no evidence for accumulation in tissues was found (NRC, 1977).

Aquatic Toxicity

Aquatic toxicity rating, TLm, 96 hr: 1-10 ppm (Fairchild, 1977).
Toxicity to fish, 96 hr TLm: 0.1-1.0 mg/l (Kemp et al., 1973).

Mutagenic Potential

In vitro mutagenesis tests:

Ames test, only weakly mutagenic; E. coli B Y Vr-B strain, induced 5-fold increases in streptomycin resistant mutants (Warren et al., 1976).

Health and Ecological Effects - ETU

NRC (1977) notes that "the occurrence of ethylenethiourea (ETU) as a major decomposition product (of maneb)...presents a potential hazard since it is goitrogenic in laboratory animals and may produce thyroid carcinoma."

Rat, oral 10 mg/kg/day: teratogenic (Khera, 1973)

Weakly mutagenic to mammals (NRC, 1977); mutagenic to Salmonella typhimurium his G46 (Shirasu et al., 1977).

Produces hepatomas in mice (NRC, 1977)

In summarizing their review of ETU the National Research Council has recommended "that very strict criteria be applied when limits for ETU in drinking water are established" (NRC, 1977). Weisburger (1977) recommends that "in view of the carcinogenicity of ethylenethiourea in two animal species, human exposure should be avoided." It should be stressed, however, that the applicability of animal studies with ETU as an estimate of human health risk has been questioned and that additional animal studies with EDBD's are in progress (Gower and Gordon, 1979). For a recent review of the health effects of ETU see Gregory, 1978.

Aquatic Effects: Manganese

Researchers have found a 50% reduction in total algal cell volume of Selenastrum at a concentration of 31 mg/L manganese (Christenson and Scherfig, 1979). Concentrations of Mn above 0.005 mg/L have been shown to have toxic effects on some algae (McKee and Wolf, 1963). A threshold concentration for a 16 hr. exposure of $KMnO_4$ to Daphnia was found to be 0.63 mg/L (Rathuen and

Cairns, 1973). Immobilization of Daphnia in Lake Erie water occurred at 50 mg/L $MnCl_2$ (McKee and Wolf, 1963). Lethal concentrations of Mn as $KMnO_4$ ranged from 3.2 to >32 mg/L for thirteen protozoan species, while tolerated concentrations ranged from 0.65 to 3.2 mg/L (Ruthven and Cairns, 1963). McKee and Wolf (1963) recommended a maximum concentration of 1.0 mg/L Mn in natural waters to prevent deleterious effects to fish and aquatic life.

Aquatic Effects: Zinc

Zinc has been shown to be algicidal to Selenastrum capricornutum at 0.7 mg/L and inhibitory to growth at 0.1 mg/L (Bartlett et al., 1974). Oxygen production by Selenastrum was reduced by 50% or more by 1 mg/L Zn (Hendricks, 1978). Sensitivity of Selenastrum to zinc was shown to be inversely proportional to increase in ionic strength of the test substrates (Greene et al., 1975). Ruthven and Cairns (1973) reported a median threshold level of 1.0 to 1.4 mg/l Zn for Scenedesmus.

Daphnia and other crustaceans have also been shown to be susceptible to zinc. Full life cycle no-effect concentrations to daphnids were shown to be similar to those for gammarids and fish, even though different metal salts, water hardness, and dilution water quality were used (Maki, 1978). McKee and Wolf (1963) reported that 1.36 mg/L $ZnCl_2$ in pond water killed Daphnia in less than 5 days and that the threshold concentration for immobilization of Daphnia in Lake Erie water was much less than 0.15 mg/L $ZnCl_2$. The median threshold level effect for Daphnia using $ZnSO_4$ was shown to be 1.8 mg/L (McKee and Wolf, 1963). Ruthven and Cairns (1973), in a review of toxicity data, reported a lethal level of 12.0 mg/L Zn for Daphnia.

In a study of Zn toxicity to rotifers (Philodina acuticornis) by Buikema, Cairns, and Sullivan, (1974) Zn toxicity was found to be similar after 96 hours, regardless of the Zn salt used. Zn toxicity decreased as hardness and alkalinity increased, with 96 hr EC_{50} values ranging from 0.3 to 31.0 mg/l depending on total hardness (0 to 291 ppm $CaCO_3$). Rotifer eggs were very resistant to Zn toxicity and young rotifers were more sensitive than adult.

Ruthven and Cairns (1973) reported lethal Zn (as $ZnSO_4 \cdot 7H_2O$) concentrations to 13 protozoan species ranging from 5.6 to greater than 1000 mg/L while tolerated concentrations ranged from 0.56 to 5000 mg/L. The LD_{50} for Zn for Vorticella campanula was 1.9 ppm while the LD_{100} was greater than 10 ppm.

Degradation of Ethylene Bisdithiocarbamates

Ethylenebisdithiocarbamates (EBDC) are unstable in the presence of moisture and oxygen as well as in biological systems. Ethylenethiourea (ETU) is not a final product but is an intermediate in the degradation of the EBDC fungicides. ETU is stable with respect to hydrolytic reactions but is easily oxidized to ethylene urea (EU). The oxidation of ETU takes place primarily in biological systems and by photolytic reactions, especially in the presence of photosensitizers (Commission on Terminal Pesticide Reviews, 1977). Some of the degradation studies with EBDC and ETU are summarized in Table 1. In addition, ETU has been shown to be stable in deionized water in the absence of photosensizers but rapidly oxidized in their presence. Samples of waters from rivers, lakes, and agricultural areas were found to degrade ETU to EU in sunlight. Degradation also occurred in the dark after prior exposure to sunlight. Natural photosensitizers, such as amino acids, acetone, riboflavin, and chlorophyll occur in water and soil. Furthermore, experiments on ETU degradation in sands exposed to sunlight showed no significant build-up of ETU after 7 days. In sterile soils, ^{14}C-ETU degradation to EU was slower compared to non-sterile soil, and no release of $^{14}CO_2$ from ETU was noted. In non-sterile soils, ^{14}C-ETU was rapidly converted (~8 days) to EU. Preliminary data from anaerobic soils show slower rates of degradation with the estimated half-life of ETU degradation (0.4 ppm) being 20-30 days.

ETU is inherently mobile in the soil as shown by soil thin-layer chromatography (Lyman, 1977) but is likely to undergo decomposition before being translocated in the soil. Unlike the parent EBDC's, ETU can be taken up by plant root systems, translocated, and metabolized. (Lyman, 1977; Commission on Terminal Pesticide Residues, 1977).

In a 1972 study of ETU levels in 167 Canadian food products, 33% of the samples contained detectable (>0.01 ppm) ETU, with most values being 0.02 ppm or less. The highest value was 0.083 ppm in orange peel (Commission on Terminal Pesticide Reviews).

Since ETU is detected as a metabolite in EBDC degradation and because of its public health implications, Sanborn et al., (1977) have not recommended soil disposal of dithiocarbamate fungicides until ETU is shown definitely to be non-persistent in soils.

Table 1. DEGRADATION OF EBDC AND ETU

Matrix	Concentration			Time	Comments	References
	Initial		Final			
	EBDC	ETU	ETU			
Pond water	--	0.1 ppm	ND	40 days		Lyman (1977)
		0.1 ppm	0.04 ppm			
Distilled water		1.0 ppm	0.7 ppm	40 days		
			No change from initial concentration	40 days		
Soils (25 locations)	Agricultural use for 6-22 years		>0.02 ppm in 4 of 25 sites maximum value 0.12 ppm		[a]	Lyman (1977)
Soil (Immokalee)		Sprayed on soil surface	68 ppm	13 days		Lyman (1977)
			trace	27 days		
Soil (Hagerstown silt loam)		10 ppm	5 ppm	~22 days	Normal CO_2 production not disturbed	Commission on Terminal Pesticide Residues (1977)
Water		1, 10, 100 ppm	0.3, 7.3, 69 ppm	30 days	Microcasm study ETU added to water[b]	Lyman (1977)
Algae			3.8, 4.5, 664 ppm	30 days		
Snails			0.8, 48.3, 151 ppm	30 days		
Daphnia			13.1, 130, 1200 ppm	30 days		
Fish			0.8, 8.2, 53.7 ppm	30 days		

[a] No ETU was found in soil from run-off pathways, water and mud from ponds or streams, or in aquatic wildlife.

[b] Organisms did accumulate ^{14}C from ^{14}C-ETU, but ETU did not appear to be biomagnified through trophic levels, at least for the short duration of the study. Only ^{14}C was determined and the chemical form was not identified.

SECTION 4

TREATABILITY STUDIES

SAMPLE COLLECTION

Collection of EBDC Wastewaters

A sampling strategy was devised in collaboration with the manufacturer which would answer questions about the effectiveness of treating isolated process wastestreams versus a mixture of many processes and be representative of actual conditions especially with respect to dilution rates. The EBDC production was at 90-100% of plant capacity during the sampling.

EBDC production accounted for 12% of the total plant effluent. Both wastewaters had the appearance of orange juice with reference to color and turbidity, however the EBDC wastewater darkened on storage. Six aqueous effluents are produced and 5 of these combine to form one wastewater stream. The major streams are the filtrate from the vacuum filtration and the washwater from product purification. The preliminary samples of EBDC wastewater were collected from 8:00 am, 1/21/80 to 8:00 am, 1/23/80 using an Isco Interval Sampler. Operation of the plant was normal and no upsets occurred. Similar sampling was performed on 2/1-4/80, and these samples were used in the full scale studies. A settling step was included before the sampling; this step represents new control methodology not in place at the time of the previous study (Little et al., 1980). For this settling to be effective it must occur before the wastestream is mixed with other wastestreams which contain surfactants. A portion of the test sample, however, was collected when surfactants were introduced before settling. The whole plant effluent wastewater was collected over the same time periods as the EBDC wastewater using a Sigma Motor peristaltic pump. This sample was lime treated, but no solids were removed.

Transport of Sample and Sludge

Sludge samples from the POTW receiving the EBDC wastewater were obtained on 23 January 1980. Two five gallon metal drums were used to transport the sludge to RTI. The time lapse between sampling and aeration at RTI was approximately 8 hours. Upon arrival at RTI the sludge was aerated and placed in an O.E.D.C. activated sludge unit and fed municipal sewage until acclimated and subsequent tests were run (vide infra).

On 7 February 1980, the wastewater samples arrived at RTI approximately 72 hours after final collection. Four five gallon glass jars of whole plant wastewater and two five gallon glass jars of EBDC wastewater were received and immediately stored at 4°C.

Characterization of Wastewaters

The EBDC and whole plant wastewaters were characterized for pesticide and ETU content and for standard wastewater parameters (Table 2). Both wastewaters were strongly alkaline and contained high levels of dissolved solids, in comparison to municipal wastewater. The following additional observations can be made: high levels of Cl^-, TKN, and COD; low phosphorus. The whole plant wastewater had a COD concentration approximately 6 times that of the EBDC wastewater, indicating that the EBDC process was contributing proportionally less to the overall COD than would be indicated by the flow volume. On the other hand, as would be expected, the EBDC wastewater had much higher levels of EBDC's and ETU.

Except for the alkalinity due to liming of these samples, these wastewaters tended to be more dilute than those collected in the previous study (Little, et al., 1980), which was focused on the final step in the EBDC production process.

Algal and Daphnia bioassays were conducted on each wastewater (Table 3). For both wastewaters, the 48 hr EC_{50} for Daphnia lay between 1 and 10%. The algal assays indicated an EC_{50} after 14 days incubation of between 1 and 10% for both the EBDC wastewater and the whole plant wastewater. The zinc concentrations (Table 4) in these untreated wastewaters by dilution would be as follows:

EBDC, 0.1%: 0.0046 mg/l

Table 2. CHARACTERIZATION OF EBDC WASTEWATERS

Parameter	EBDC Composite	Whole Plant Composite
pH	10.1	10.5
Cl^-, mg/L	391 ± 14	707 ± 14
Total Kjeldahl Nitrogen, mg/L as N	394	114
Ammonia, mg/L as N	323	86
Nitrate - Nitrite, mg/L as N	1.0	7.0
Organic Nitrogen, mg/L as N	71	28
Total Phosphorus, mg/L	<0.05	0.89
Chemical oxygen demand (COD), mg/L	510	3106
Alkalinity mg/L $CaCO_3$	1180	1620
Total Solids mg/L	12,820	9240
Dissolved Solids mg/L	12,360	8160
Ethylenebisdithiocarbamate and its salts[a], mg/L	41.6	16.0
ETU, mg/L	149.0	22.4

[a]Maneb was used as the standard.

Table 3. EFFECT OF EBDC WASTEWATERS ON ALGAL GROWTH AND DAPHNIA SURVIVAL

Test Assay	Wastewater Conc., % v/v				
	0	0.01	0.1	1.0	10.0
(1) EBDC					
Algal Growth (% of control at day 14)	100	109	93	84	31
Daphnia magna (survival at 48 hr., %)	90	-	100	80	0
(2) Whole Plant Wastewater					
Algal Growth	100	-	86	52	4
Daphnia magna (survival at 48 hr. %)	100	-	100	100	0

Table 4. ATOMIC ABSORPTION DETERMINATION OF METALS IN EBDC WASTEWATER[a]

Sample	Zn (mg/1)	Mn (mg/1)
EBDC Wastewater with surfactant	6.40	-
EBDC Wastewater without surfactant	1.68	-
EBDC Wastewater composite	4.60	1.70
Whole plant wastewater	1.20	1.40
Control influent/O.E.C.D. Unit	<0.1	-
Control effluent/O.E.C.D. Unit	<0.1	-
2.5% Whole plant influent/O.E.C.D. Unit	<0.1	-
2.5% Whole plant effluent/O.E.C.D. Unit	<0.1	-
0.25% EBDC influent/O.E.C.D. Unit	<0.1	-
0.25% EBDC effluent/O.E.C.D. Unit	<0.1	-

[a] The analyses were performed during the period of treatment and biotesting. No attempt was made to preserve the samples to reflect the concentration at the manufacturing facility but to reflect the sample being tested.

1.0%: 0.046 mg/l

10.0%: 0.46 mg/l

Whole plant, 0.1%: 0.0012 mg/l

1.0%: 0.012 mg/l

10.0%: 0.12 mg/l

Zn toxicity to Daphnia and algae is known to be strongly influenced by water quality, especially hardness. Levels as low as 0.15 mg/l of $ZnCl_2$ have been reported to immobilize Daphnia, while levels as low as 0.1 mg/L of Zn have inhibited algal growth (see literature review, Section 3). It is therefore possible that toxicity of these wastewaters is due in part to their Zn content.

TREATMENT OF EBDC WASTEWATERS IN BENCH SCALE ACTIVATED SLUDGE UNITS

Whole plant and EBDC production wastewaters were diluted to 2.5 and 0.25% (v/v) respectively, with municipal sewage and fed to bench-scale activated sludge (AS) units. These concentrations were chosen to reflect the actual dilution of the wastewaters in the POTW plant which receives the plant wastes. The wastewaters were subjected to primary settling prior to AS treatment.

As described in Appendix B, two types of bench scale units were employed in these tests. The "Swisher units", originally designed for surfactant degradation studies (Swisher, 1970), were utilized in previous pesticide wastewater studies (Little et al., 1980). The somewhat larger O.E.C.D. units (Organization of Economic Cooperation and Development, Bundegesetzblatt, 1977) were also utilized in this study on the recommendation of the EBDC manufacturer. The O.E.C.D. test units (i.e., those to be fed pesticide wastewaters) were initially inoculated with sludge collected from the POTW receiving the plant wastewaters. The rationale for this action was that this sludge would already be acclimated to the wastewater and would possibly degrade the pesticide more rapidly. The Swisher units were inoculated with sludge collected from the Hope Valley Sewage Treatment facility in Durham, North Carolina, which treats largely domestic wastewater. The O.E.C.D. control unit was inoculated with Hope Valley sludge.

Measurements of the pH were taken each day in the aerator section of the activated sludge units. From February 15, 1980 both the Swisher and O.E.C.D. control units received the same feed which was buffered as required by the

O.E.C.D. control unit. Buffering was used for the test units where the pH fell below 6.5 for 2 to 3 days. Sodium bicarbonate (0.4 g/L) was added to the influent of AS units to be buffered.

Buffering of influents was initiated in the units on the following dates:

Unit	Date
O.E.C.D. Control	5 February 1980
Swisher Control	15 February 1980
Swisher EBDC	26 February 1980
Swisher Whole Plant	26 February 1980
O.E.C.D. EBDC	6 March 1980

Operating characteristics of the bench-scale plants are shown in Tables 5-13 and Figures 1-6. Aerator dissolved oxygen levels were high and more than adequate to permit aerobic degradation of organic materials, as well as nitrification. Due to buffer addition, pH levels were generally close to neutral. Periodic tests of oxygen consumption confirmed the viability of the sludges.

Previous studies in which concentrated EBDC production wastewater was fed to AS units at a 10% (v/v) dilution showed a somewhat, although small, adverse effect on ability of the units to remove COD (Little et al., 1980). The present studies, reflecting actual wastewater concentrations at the POTW, indicated that COD removals generally were unaffected by the presence of either wastewater (Figure 7-14, Tables 14-16), especially after the initial start-up. COD removals in control units of the two types of reactors were strikingly similar (Table 17). However, in the test units the COD removals in the Swisher units tended to be somewhat greater than in the O.E.C.D. units (Tables 14-16), possibly reflecting the superior ability of the Hope Valley sludge to degrade the wastewater.

The previous study (Little et al., 1980) gave some indication that EBDC wastewater might inhibit nitrification. Therefore, in the present study more extensive nitrogen analyses of the units' influents and effluents were undertaken. The results (Table 18) indicate that substantial nitrification (conversion of ammonia to nitrite/nitrate) occurred in the control units but was inhibited in units receiving either wastewater. Some small amount of nitrification took place in the Swisher test units, but not in the O.E.C.D. units, raising the possibility that the POTW sludge had already lost its nitrifying ability while the Hope Valley sludge still contained a few viable nitrifiers.

Table 5. OPERATIONAL CHARACTERISTICS OF SWISHER ACTIVATED SLUDGE UNITS--CONTROL UNITS

Date	Dissolved O_2 (mg/L)	Temp. (^oC)	pH	Solids Wasted mL	O_2 Consumption (mg/g/hr)
1-30					
1-31					
2-01					
2-04					
2-05					
2-07					
2-08					
2-11					
2-12					
2-13	3.9	24.9			
2-14	4.6		7.11	10	
2-15	5.6	25.9	7.29		
2-18	4.9	21.2			
2-19	3.8	26.1	7.31	20	
2-20	4.9	21.2			
2-21	3.4	28.0	7.32	10	
2-22	3.9	28.3	7.24	20	17.6
2-25	5.0	23.0	7.23	10	
2-26	5.2	22.0	7.31	40	
2-27	4.8	22.0	6.89	20	
2-28	4.5	23.0	7.33	30	
2-29	4.3	25.3	7.41	20	
3-04	5.3	22.9	7.32	10	
3-05	5.3	23.6	7.50	20	
3-06	5.7	-	7.45	20	
3-07	6.2	23.2	7.45	-	
3-10	6.0	22.2	7.40	-	

Table 6. OPERATIONAL CHARACTERISTICS OF SWISHER ACTIVATED SLUDGE UNITS--
0.25% EBDC WASTEWATER SERIES

Date	Dissolved O_2 (mg/l)	Temp. (°C)	pH	Solids Wasted mL	O_2 Consumption (mg/g/hr)
1-30					
1-31					
2-01					
2-04					
2-05					
2-07					
2-08					
2-11					
2-12					
2-13	5.5	24.9			
2-14	5.3		7.29	10	
2-15	7.1	25.5	7.52		
2-18	7.3	21.1			
2-19	5.6	26.2	7.59	20	
2-20	7.3	21.1			
2-21	6.5	27.3	7.60	10	6.85
2-22	6.6	28.0	7.38		
2-25	7.2	23.0	5.37		
2-26	6.1	22.2	5.48	30	
2-27	6.3	22.2	7.62	20	
2-28	5.5	23.0	7.60	20	
2-29	5.4	25.5	7.58	20	
3-04	6.4	22.9	7.40	10	
3-05	6.0	23.6	7.51	20	
3-06	6.2	-	7.40	20	
3-07	6.7	23.2	7.50	-	
3-10	6.6	22.2	7.40	-	

Table 7. OPERATIONAL CHARACTERISTICS OF SWISHER ACTIVATED SLUDGE UNITS--
2.5% WHOLE PLANT WASTEWATER SERIES

Date	Dissolved O_2 (mg/L)	Temp. (°C)	pH	Solids Wasted mL	O_2 Consumption (mg/g/hr)
1-30					
1-31					
2-01					
2-04					
2-05					
2-07					
2-08					
2-11					
2-12					
2-13	4.7	24.9			
2-14	5.3		7.02	10	
2-15	6.8	25.9	7.50		
2-18	6.4	21.1			
2-19	4.9	26.0	7.41	20	
2-20	6.4	21.1			
2-21	4.7	28.0	7.43	10	6.45
2-22	4.9	28.0	7.35	20	
2-25	5.0	23.0	7.23	10	
2-26	3.2	22.2	5.51	40	
2-27	4.0	22.1	7.30	20	
2-28	2.9	23.0	7.52	30	
2-29	4.7	25.5	7.49	20	
3-04	5.4	22.9	7.25	10	
3-05	5.4	23.6	7.50	20	
3-06	5.8	-	7.40	20	
3-07	6.2	23.2	6.92	-	
3-10	6.5	22.2	7.68	-	

Table 8. OPERATIONAL CHARACTERISTICS OF O.E.C.D. ACTIVATED SLUDGE UNITS--CONTROL

Date	Dissolved O_2 (mg/L)	Temp. (^{o}C)	pH	Solids Wasted mL	O_2 Consumption (mg/g/hr)
1-30	6.3	17.2	6.30	20	
1-31	7.3	17.3	6.48		
2-01	6.5	17.0	5.95		
2-04	8.6	17.2	6.48		
2-05	6.8	16.2	5.30	10	
2-07	8.2	17.8	7.42		
2-08	8.5	17.0	7.82	30	
2-11				20	
2-12	6.4	18.2	7.45		
2-13	7.3	18.0	7.75		
2-14	3.8		7.20	10	
2-15	7.5	19.0	7.27		
2-18	4.6	17.2			
2-19	5.0	17.0	7.19	10	
2-20	4.6	17.2			
2-21	4.4	21.2	7.20	10	12.4
2-22	4.3	21.4	7.18	100	
2-25	4.8	20.0	7.22	100	
2-26	5.4	16.5		100	
2-27	3.8	16.0	7.28	100	
2-28	4.8	16.5	7.25	240	
2-29	6.3	17.0	7.28	300	
3-04	5.9	18.0	7.21	10	
3-05	7.4	18.0	7.52	10	
3-06	8.5	-	7.72	300	
3-07	8.4	17.2	7.40	10	

Table 9. OPERATIONAL CHARACTERISTICS OF O.E.C.D. ACTIVATED SLUDGE UNITS--0.25% EBDC WASTEWATER SERIES

Date	Dissolved O_2 (mg/L)	Temp. (^oC)	pH	Solids Wasted mL	O_2 Consumption (mg/g/hr)
1-30	7.7	18.0	7.21	20	
1-31	8.3	17.0	7.45		
2-01	6.6	16.9	7.22		
2-04	8.7	17.2	7.32		
2-05	7.4	17.0	7.41	10	
2-07	9.2	17.2	7.48		
2-08	8.8	16.4	7.60	30	
2-11				20	
2-12	7.6	17.8	7.38		
2-13	8.0	18.2	7.62		
2-14	7.3		7.55	10	
2-15	6.7		7.52		
2-18	7.0	17.0			
2-19	6.6	17.0	7.51	20	
2-20	7.0	17.1			
2-21	6.8	21.2	7.42	10	14.9
2-22	4.4	21.3	7.40		
2-25	6.2	20.0	7.40		
2-26	7.7	16.0	7.21		
2-27	7.7	15.5	7.32		
2-28	7.7	16.0	7.30	10	
2-29	7.5	17.0	7.30		
3-04	4.8	17.5	6.87	10	
3-05	3.8	17.3	6.62	10	
3-06	3.4	-	6.35	10	
3-07	6.6	17.2	7.20	10	

Table 10. OPERATIONAL CHARACTERISTCS OF O.E.C.D. ACTIVATED SLUDGE UNITS--
2.5% WHOLE PLANT WASTEWATER SERIES

Date	Dissolved O_2 (mg/L)	Temp. (°C)	pH	Solids Wasted mL	O_2 Consumption (mg/g/hr)
1-30	7.5	17.2	7.20	20	
1-31	8.4	16.8	7.50		
2-01	6.4	16.5	7.22		
2-04	8.7	17.0	7.20		
2-05	6.4	17.0	7.19	10	
2-07	8.3	17.2	7.38		
2-08	8.4	16.7	7.52	30	
2-11				20	
2-12	7.1	17.8	7.33		
2-13	8.3	18.2	7.62		
2-14	6.1		7.41	10	
2-15	7.5	19.0	7.27		
2-18	6.3	17.0			
2-19	6.8	16.2	7.42	20	
2-20	6.3	17.0			
2-21	6.6	21.2	7.49	10	10.1
2-22	6.2	21.2	7.52		
2-25	7.2	20.0	7.50		
2-26	7.6	15.8	7.25		
2-27	7.4	15.4	7.38		
2-28	7.7	16.0	7.40	10	
2-29	7.3	17.0	7.40		
3-04	8.0	17.0	7.38	10	
3-05	8.0	17.0	7.35	10	
3-06	7.2	-	7.31	10	
3-07	8.2	17.0	7.00	10	

Table 11. OPERATIONAL PARAMETERS OF ACTIVATED SLUDGE UNITS, TOTAL AND VOLATILE SOLIDS--CONTROL UNITS

Analysis Date	Swisher Units		O.E.C.D. Units		Percent Volatile Solids	
	MLSS mg/L	MLVSS mg/L	MLSS mg/L	MLVSS mg/L	SWISHER	O.E.C.D.
1-30-80			7.50	4.08		54
2-05-80*			5.61	3.22		57
2-08-80*			4.50	2.62		58
2-11-80			5.50	2.72		49
2-14-80	6.38	3.53	4.85	2.68	55	55
2-21-80*	6.25	3.70	5.37	2.10	59	58
2-26-80	6.12	3.75	4.79	3.08	61	64
2-28-80*	5.37	3.26	5.02	3.12	61	62
3-06-80*	3.87	2.58	2.35	1.53	67	65
3-10-80*	3.22	2.09	2.40	1.70	65	71

*Dates influent and effluent samples were taken for ETU analyses.

Table 12. OPERATIONAL PARAMETERS OF ACTIVATED SLUDGE UNITS, TOTAL AND VOLATILE SOLIDS 0.25% EBDC SERIES

Analysis Date	Swisher Units MLSS mg/L	Swisher Units MLVSS mg/L	O.E.C.D. Units MLSS mg/L	O.E.C.D. Units MLVSS mg/L	Percent Volatile Solids SWISHER	Percent Volatile Solids O.E.C.D.
1-30-80*			1.95	1.60		82
2-05-80*			1.47	0.97		66
2-08-80			1.23	0.88		72
2-11-80*			1.11	0.67		60
2-14-80	5.64	3.01	0.82	0.46	53	56
2-21-80*	5.18	3.10	1.87	1.30	60	70
2-26-80	5.05	3.21	2.31	1.65	64	71
2-28-80*	4.40	2.76	2.20	1.55	63	70
3-06-80*	3.79	2.47	2.59	1.89	65	73
3-10-80*	3.39	2.14	2.24	1.34	63	60

*Dates influent and effluent samples were taken for ETU analyses.

Table 13. PERFORMANCE OF ACTIVATED SLUDGE UNITS, TOTAL AND VOLATILE SOLIDS--2.5% WHOLE PLANT WASTEWATER SERIES

Analysis Date	Swisher Units		O.E.C.D. Units		Percent Volatile Solids	
	MLSS mg/L	MLVSS mg/L	MLSS mg/L	MLVSS mg/L	SWISHER	O.E.C.D.
1-30-80			2.70	2.17		80
2-05-80*			2.44	1.61		66
2-08-80			2.46	1.67		68
2-11-80*			2.20	1.37		62
2-14-80	6.33	3.59	2.14	1.34	57	63
2-21-80*	6.08	2.60	2.67	1.80	59	67
2-26-80	6.37	3.95	2.64	1.84	62	70
2-28-80*	5.38	3.23	2.74	1.83	60	67
3-06-80*	4.70	3.07	1.95	1.43	65	73
3-10-80*	3.80	2.39	1.76	1.48	63	84

*Dates influent and effluent samples were taken for ETU analyses.

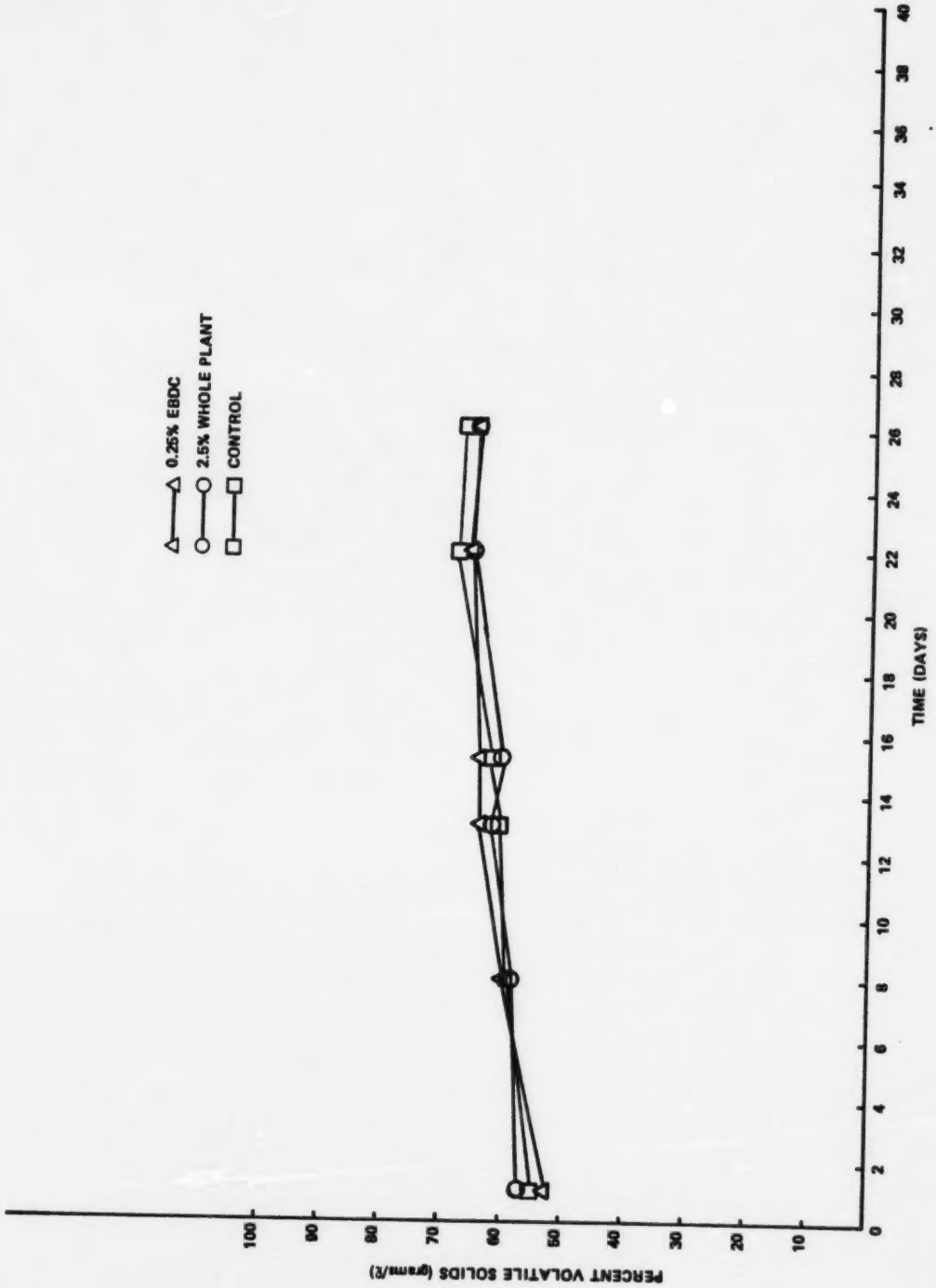


Figure 1. % Volatile Solids with Time in Swisher Units.

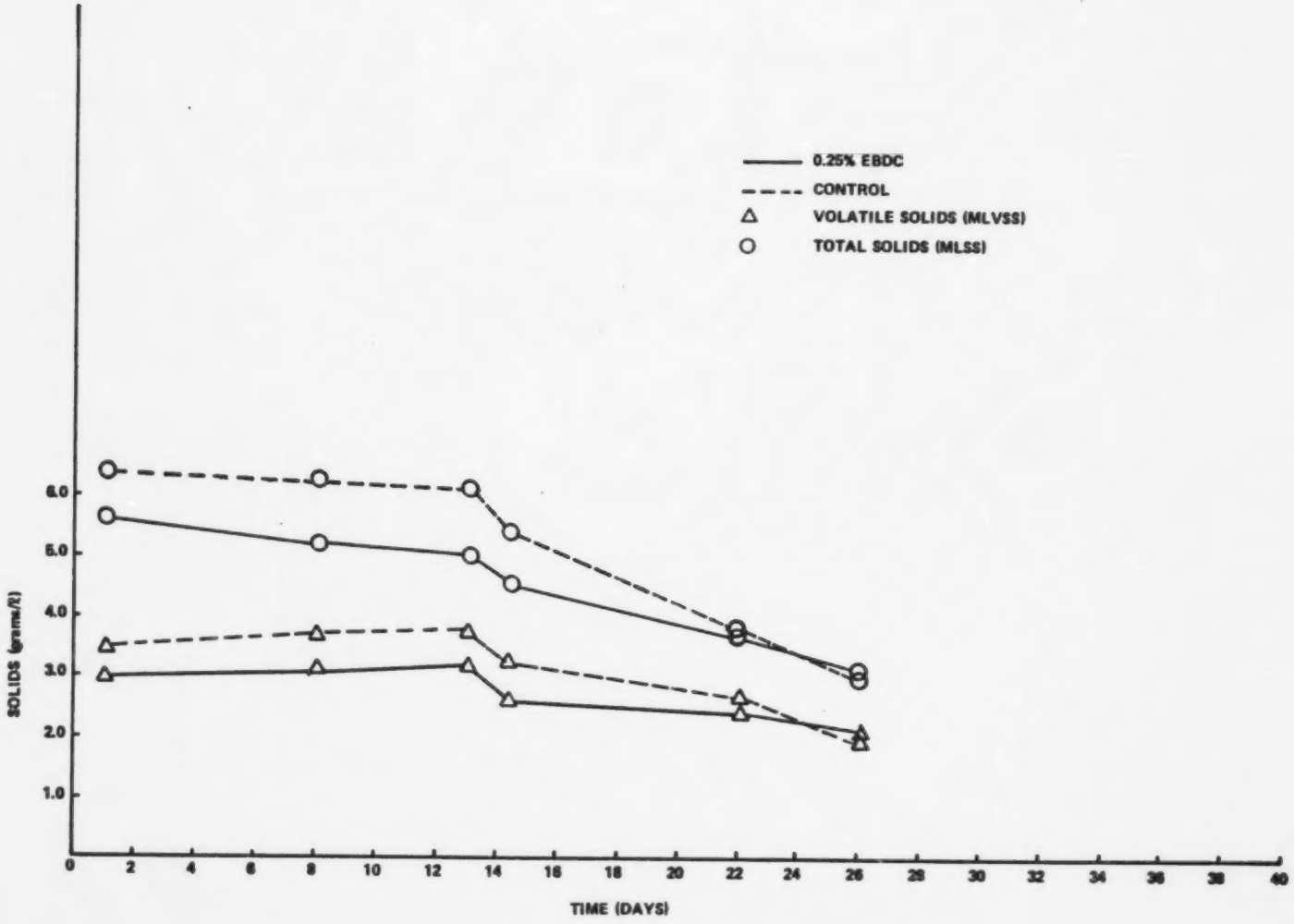


Figure 2. Total Solids and Volatile Solids in Swisher Unit Treating 0.25% EBDC Wastewater.

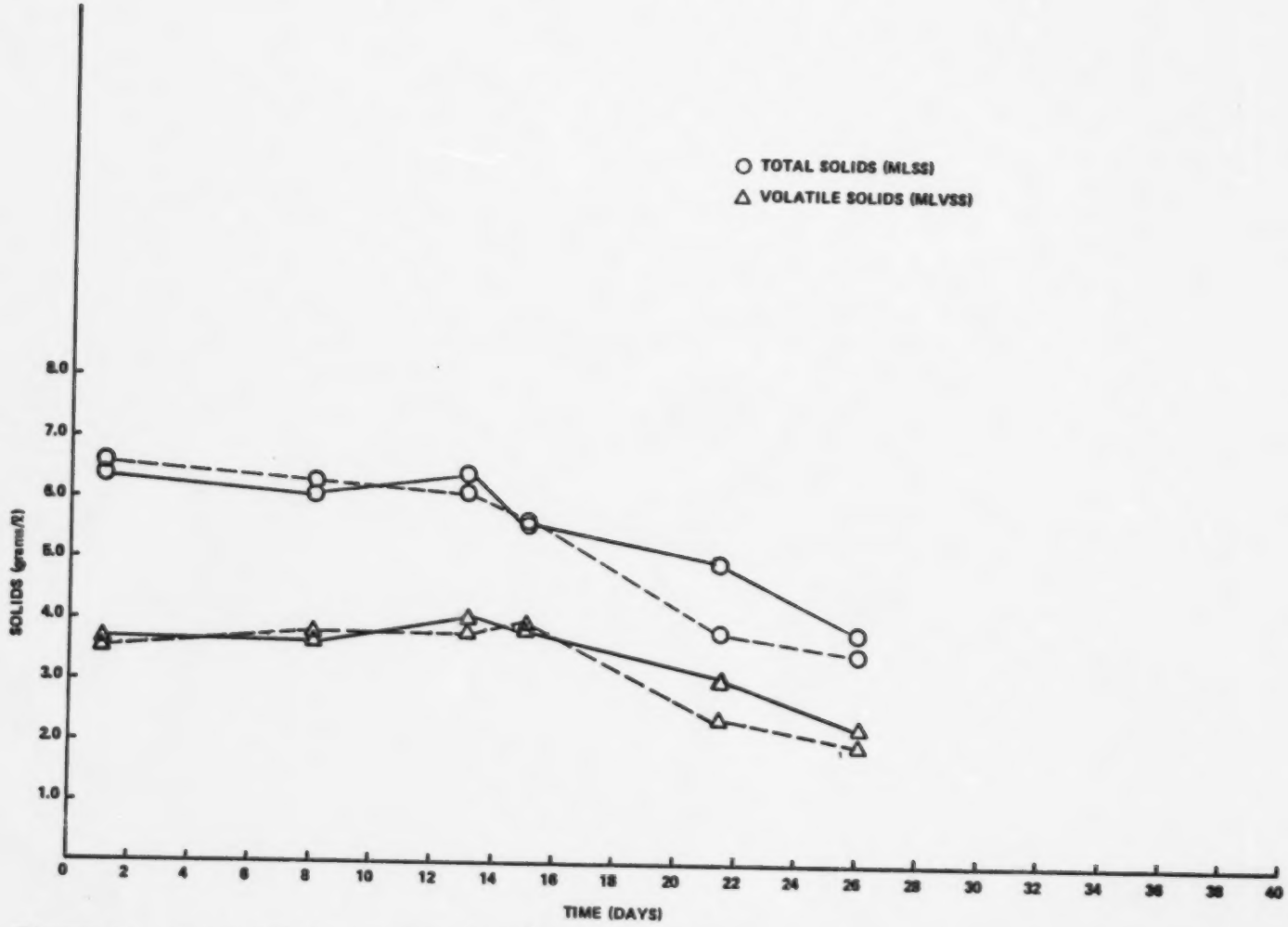


Figure 3. Total Solids and Volatile Solids in Swisher Unit Treating 2.5% Whole Plant Wastewater.

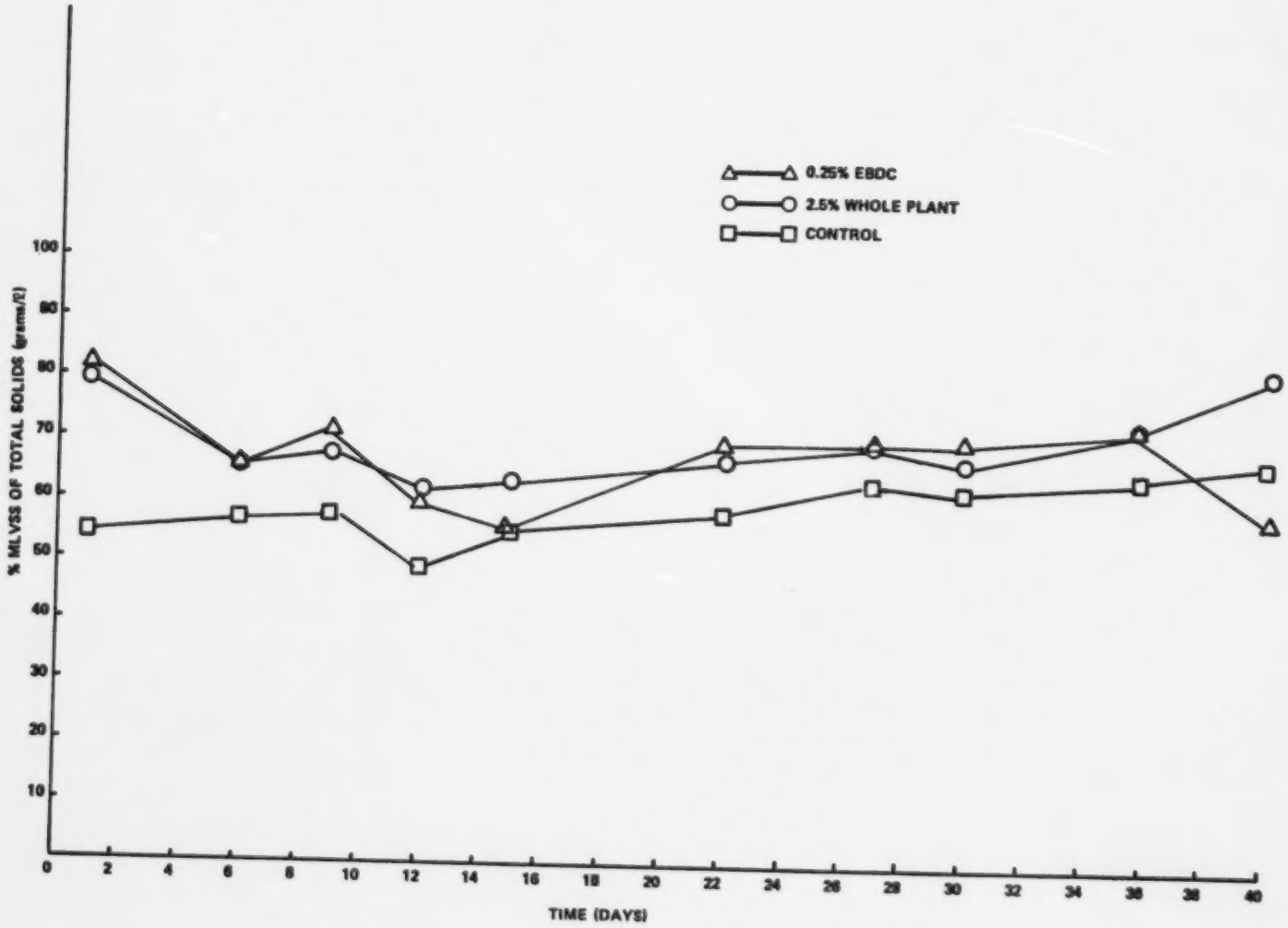


Figure 4. % Volatile Solids with Time in O.E.C.D. Units.

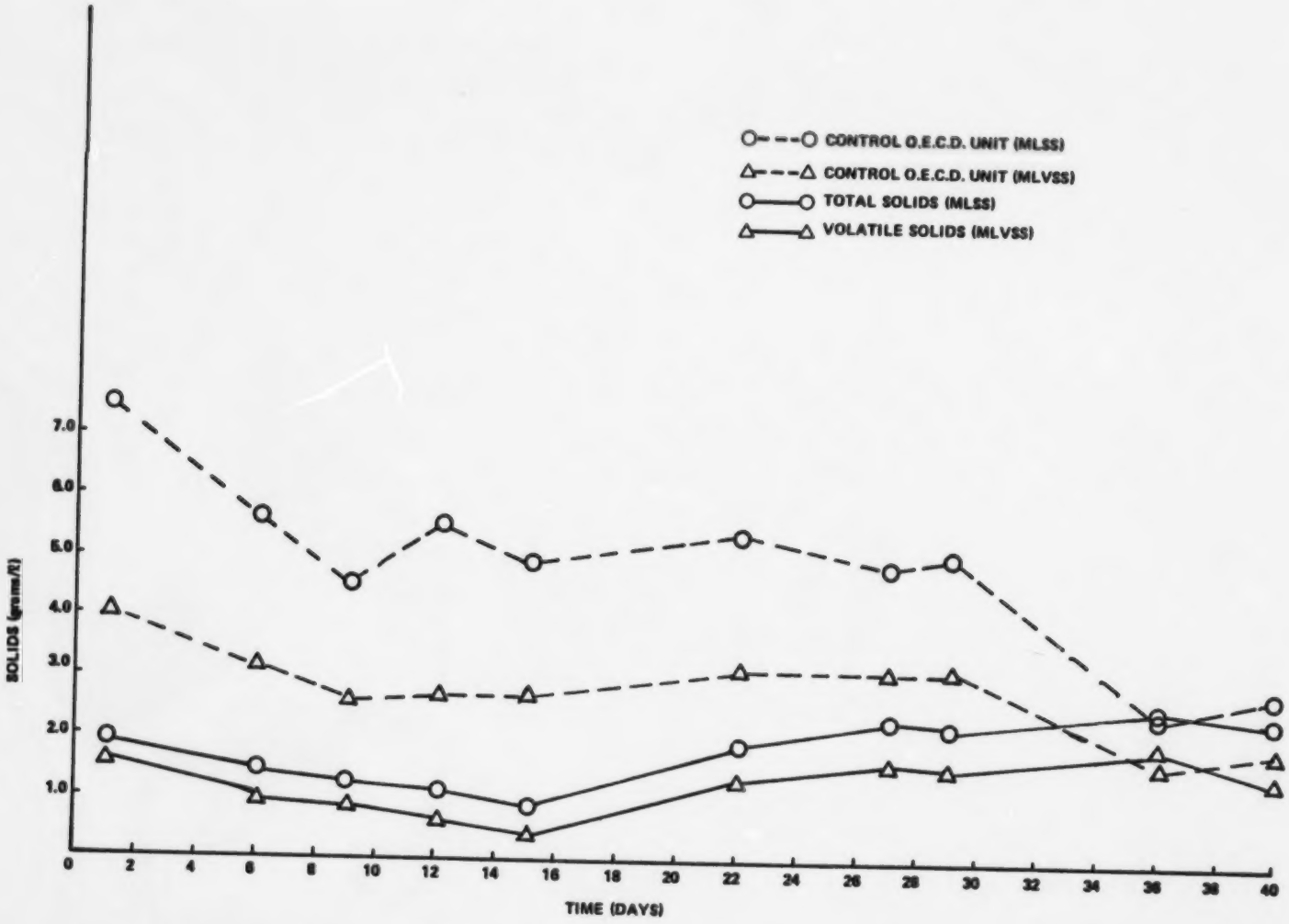


Figure 5. Total Solids and Volatile Solids in O.E.C.D. Unit Treating 0.25% EBDC Wastewater.

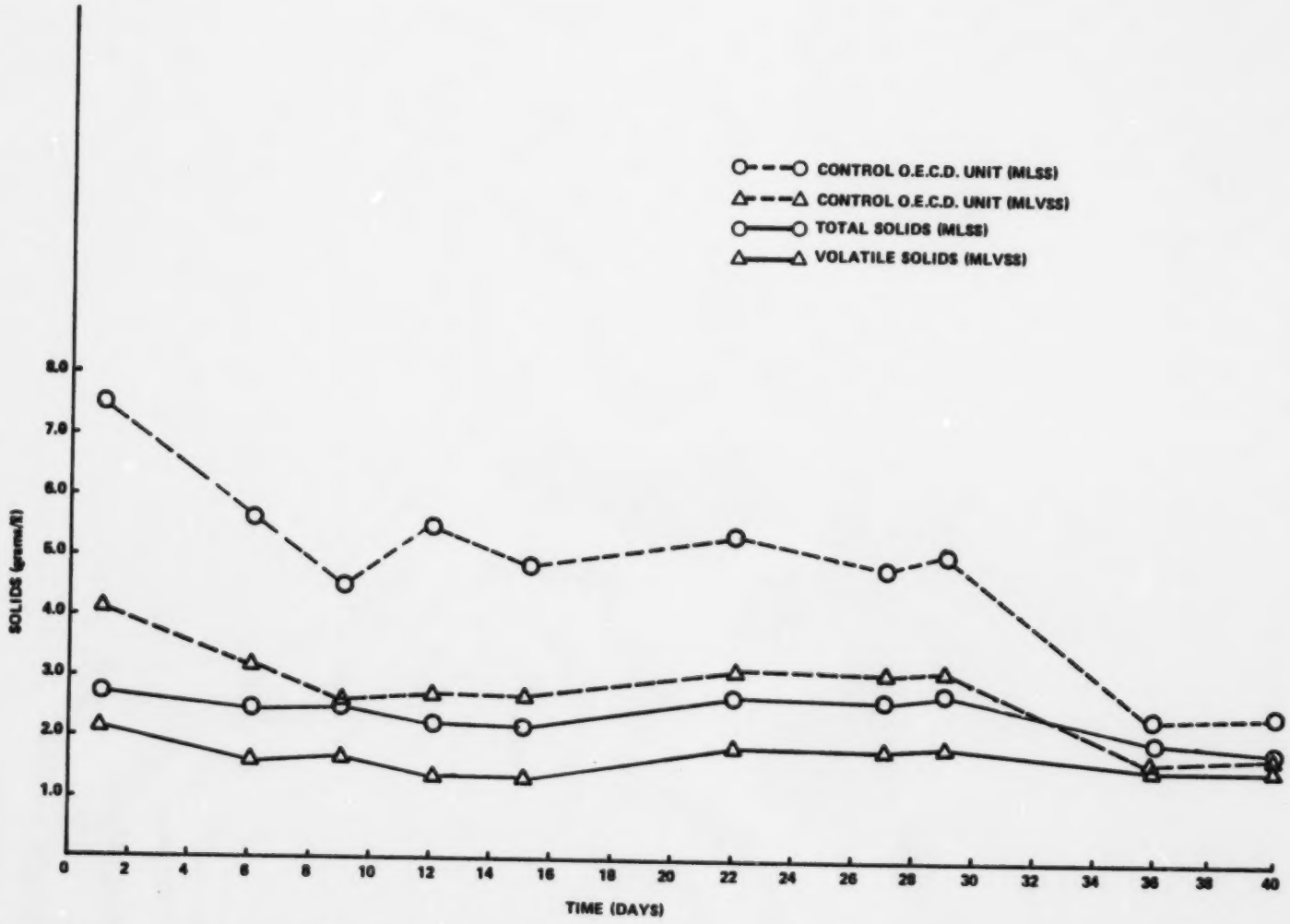


Figure 6. Total Solids and Volatile Solids in O.E.C.D. Unit Treating 2.5% Whole Plant Wastewater.

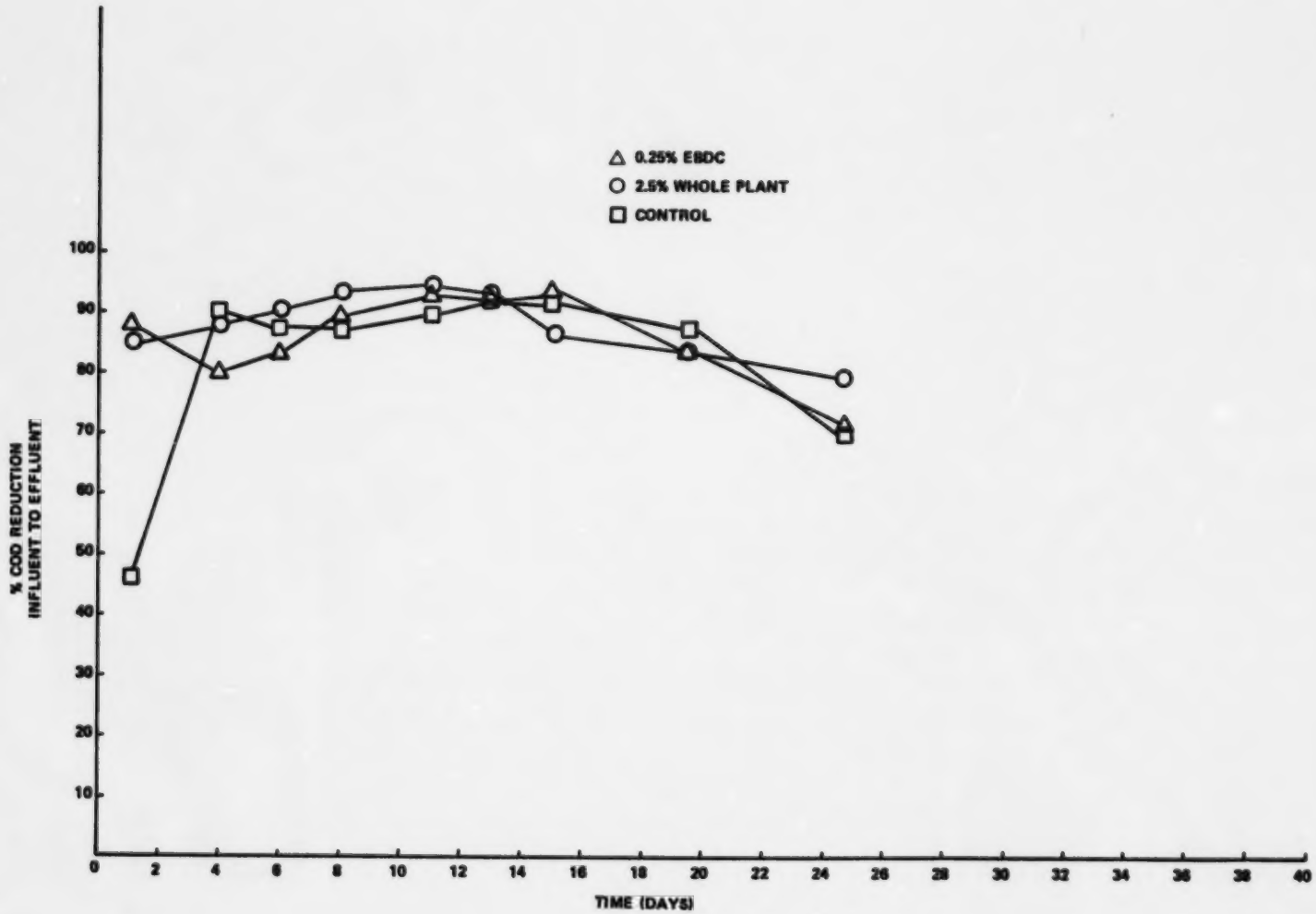


Figure 7. % COD Removal with Time in Swisher Units.

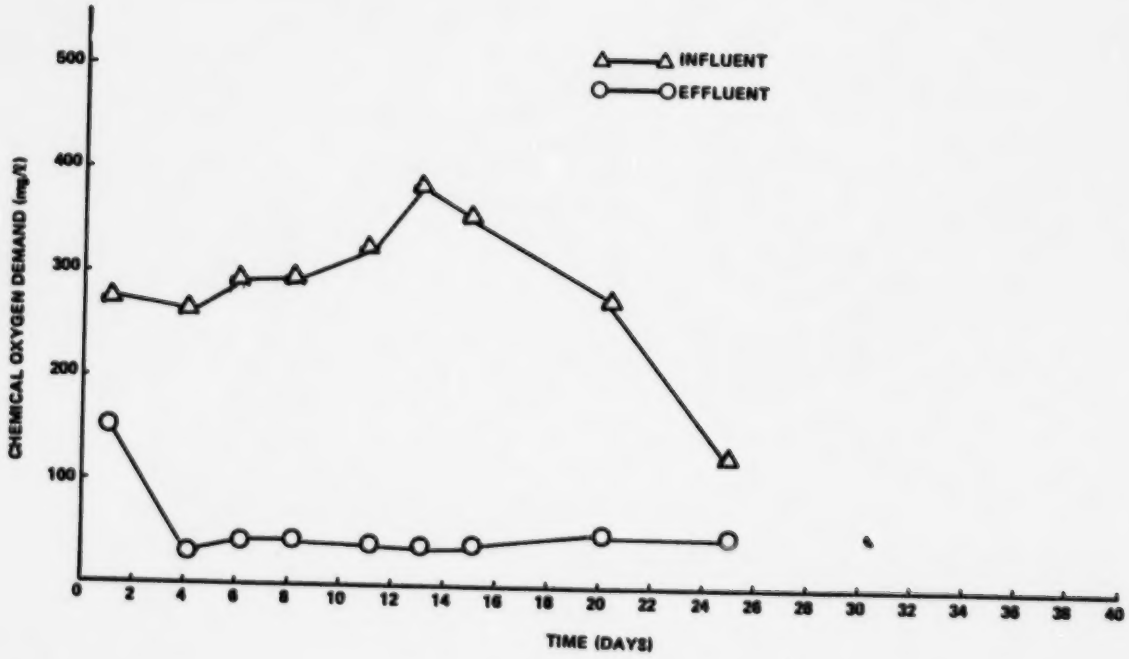


Figure 8. COD Removal in Swisher Unit Treating Municipal Sewage.

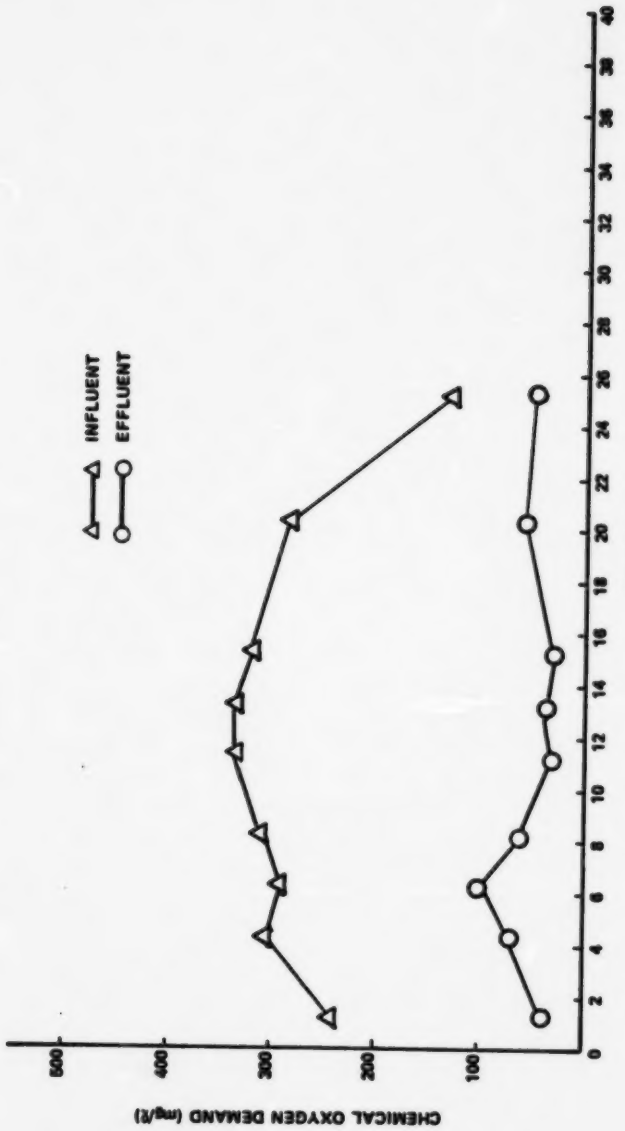


Figure 9. COD Removal in Swisher Unit Treating 0.25% EBDC Wastewater.

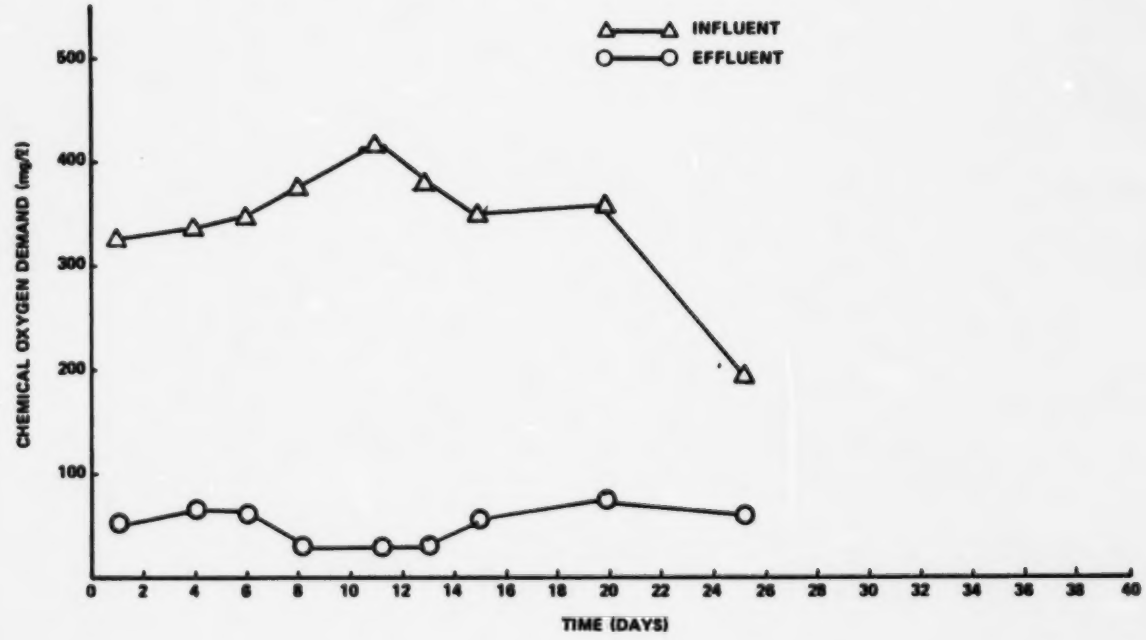


Figure 10. COD Removal in Swisher Unit Treating 2.5% Whole Plant Wastewater.

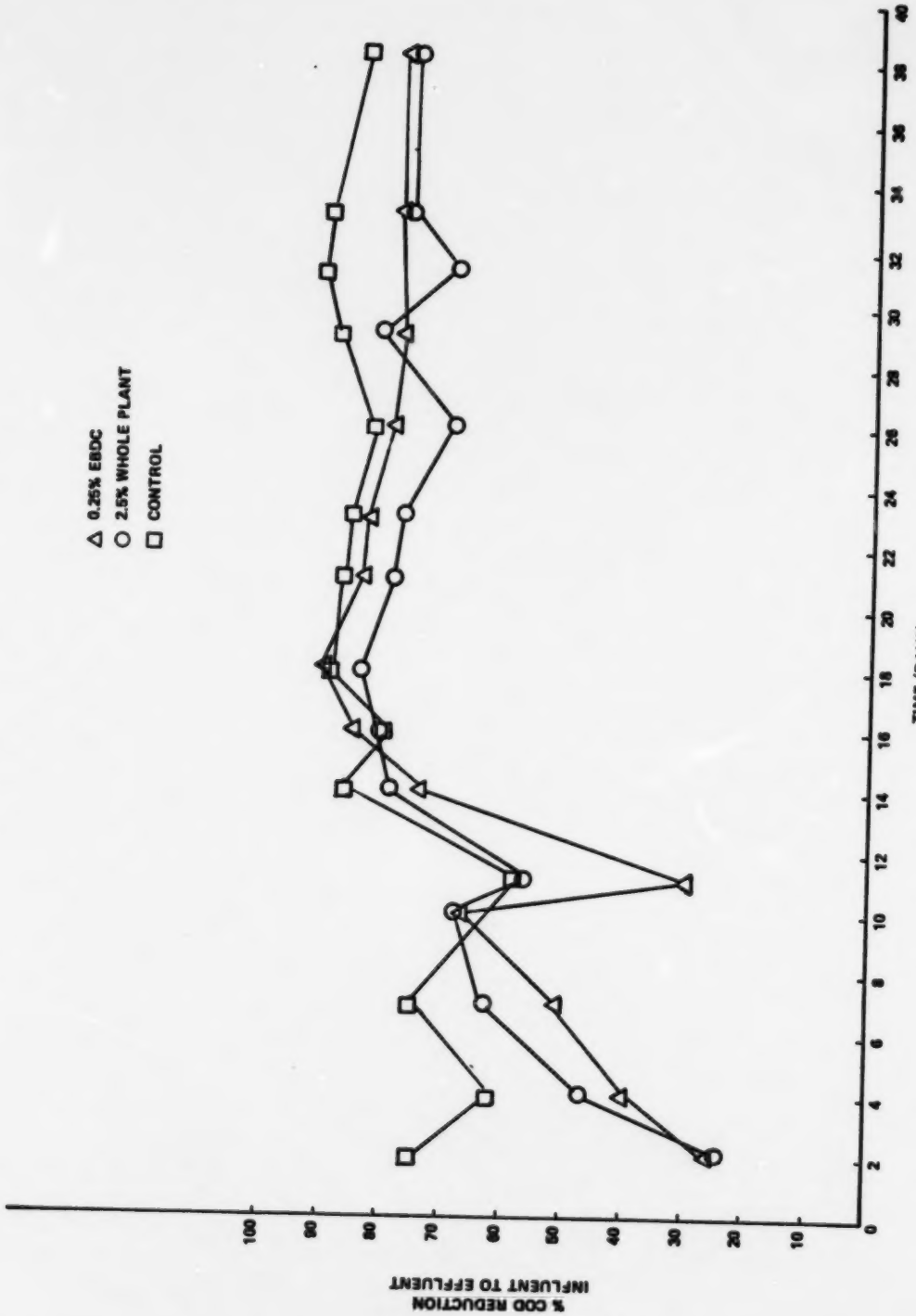


Figure 11. % COD Removal with Time in O.E.C.D. Units.

Figure 12. COD Removal in O.E.C.D. Unit Treating Municipal Sewage.

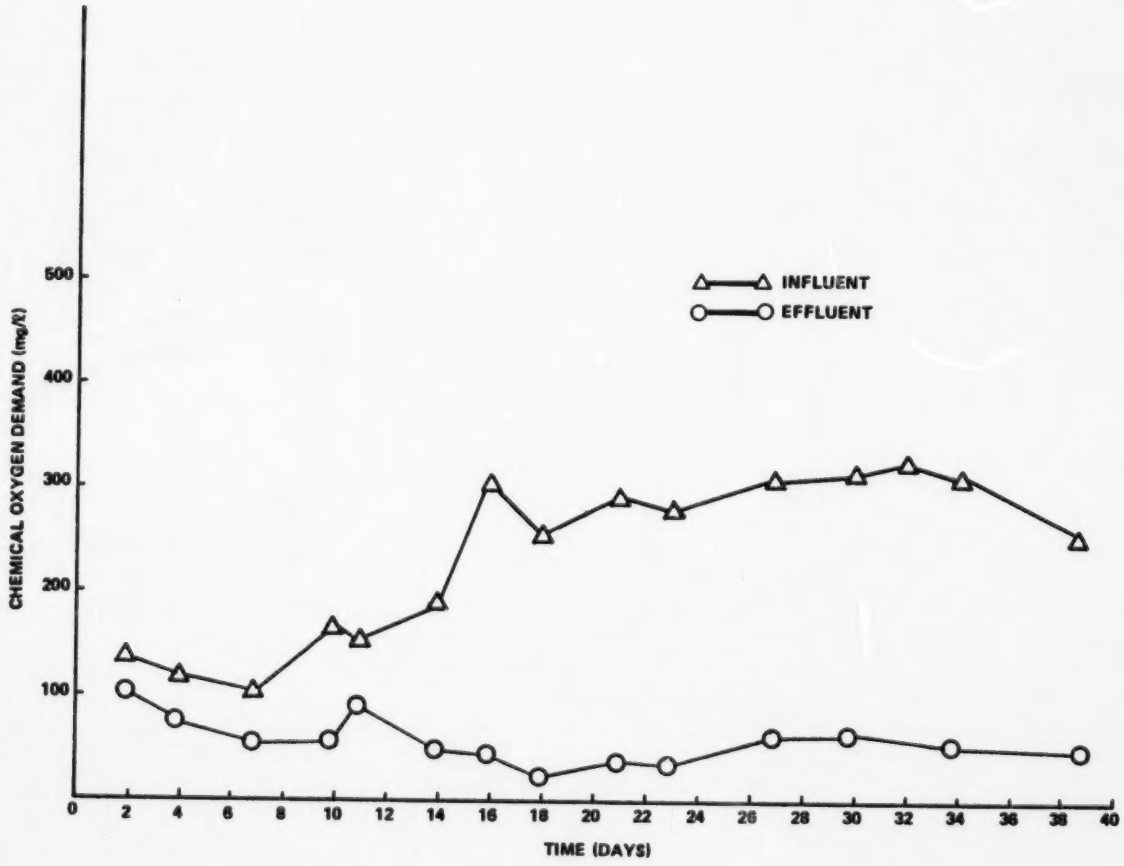


Figure 13. COD Removal in O.E.C.D. Unit Treating 0.25% EBDC Wastewater.

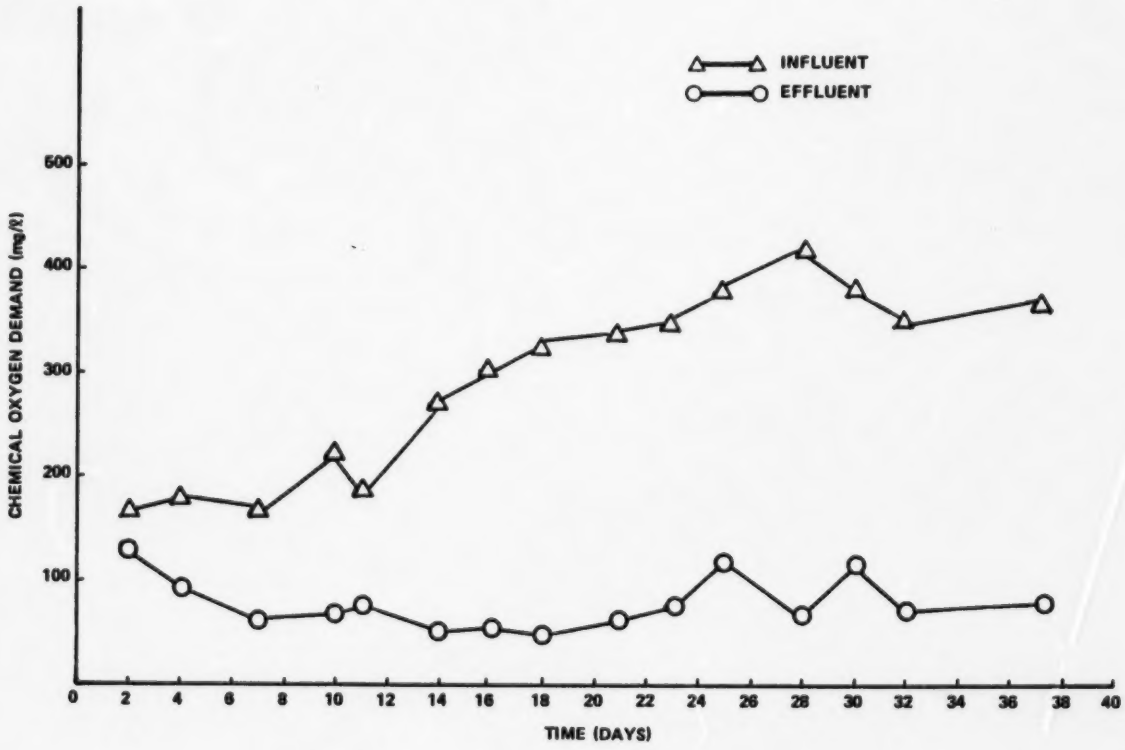


Figure 14. COD Removal in O.E.C.D. Unit Treating 2.5% Whole Plant Wastewater.

Table 14. PERFORMANCE OF ACTIVATED SLUDGE UNITS, CONTROL UNITS: COD REMOVAL

Analysis Date	COD (mg/L)				% C.O.D. Reduction	
	Swisher Units		O.E.C.D. Units			
	Influent C.O.D.	Effluent C.O.D.	Influent C.O.D.	Effluent C.O.D.	Swisher	O.E.C.D.
1-30-80			132	33.0		75
2-01-80			107	39.9		63
2-04-80			111	27.8		75
2-07-80*			179	-		-
2-08-80			117	52.0		56
2-11-80			147	20.5		87
2-13-80*			182	26.8		80
2-15-80	273	148	273	28.9	46	89
2-18-80*	260	26.9	260	32.7	90	87
2-20-80	286	37.8	286	41.7	87	85
2-22-80	288	36.7	288	51.0	87	82
2-25-80	333	34.3	333	40.4	89	88
2-27-80*	379	31.7	379	35.7	92	91
2-29-80	350	33.8	350	35.8	90	90
3-05-80*	259	37.5	259	39.5	86	85
3-10-80*	113	35.2	113	64.5	69	43

*Dates influent and effluent samples were taken for E.T.U. analyses.

Table 15. PERFORMANCE OF ACTIVATED SLUDGE UNITS, 0.25% EBDC WASTEWATER SERIES: COD REMOVAL

Analysis Date	COD (mg/L)				% C.O.D. Removal	
	Swisher Units		O.E.C.D. Units			
	Influent	Effluent	Influent	Effluent	Swisher	O.E.C.D.
1-30-80			136	101.0		26
2-01-80			121	72.5		40
2-04-80			106	52.0		51
2-07-80*			168	56.0	-	67
2-08-80			128	89.0		30
2-11-80			188	48.4		74
2-13-80*			310	46.5		85
2-15-80	258	28.9	258	25.0	89	90
2-18-80*	294	59.6	294	48.1	80	84
2-20-80	282	97.4	282	47.7	83	83
2-22-80	306	34.7	306	63.2	89	79
2-25-80	327	24.2	327	70.7	93	78
2-27-80*	329	27.8	329	-	92	-
2-29-80	312	23.9	312	67.6	92	78
3-05-80*	271	49.4	271	55.3	82	80
3-10-80*	129	39.1	129	45	70	65

*Dates influent and effluent samples were taken for ETU analyses.

Table 16. PERFORMANCE OF ACTIVATED SLUDGE UNITS, 2.5% WHOLE PLANT WASTEWATER SERIES: COD REMOVAL

Analysis Date	COD (mg/L)				% C.O.D. Reduction	
	Swisher Units		O.E.C.D. Units			
	Influent	Effluent	Influent	Effluent	Swisher	O.E.C.D.
1-30-80			172	130.0		24
2-01-80			183	94.2		49
2-04-80			169	63.1		63
2-07-80*			229	72.8		68
2-08-80			173	78.0		55
2-11-80			270	55.9		79
2-13-80*			306	58.1		81
2-15-80	324	48.2	324	52.0	85	84
2-18-80*	335	38.5	335	67.3	89	80
2-20-80	346	33.8	346	79.5	90	77
2-22-80	373	24.5	373	116.3	93	69
2-25-80	414	26.3	414	74.7	94	82
2-27-80*	375	25.8	375	115.0	93	69
2-29-80	340	49.7	340	77.5	85	77
3-05-80*	356	65.2	356	83.0	82	77
3-10-80*	194	46.9	194	88	76	55

*Dates influent and effluent samples were taken for ETU analyses.

Table 17. COMPARATIVE PERFORMANCE OF CONTROL O.E.C.D. AND SWISHER ACTIVATED SLUDGE UNITS: COD REDUCTION

Date	% COD REDUCTION	
	Swisher	O.E.C.D.
2-18-80	90	87
2-20-80	87	85
2-22-80	87	82
2-25-80	89	88
2-27-80	92	91
2-29-80	90	90
3-05-80	86	85

Table 18. EVALUATION OF NITROGEN BALANCE IN THE ACTIVATED SLUDGE TREATMENT UNITS

Date	Unit/Stream	Influent mg/L			Effluent mg/L		
		Organic N	NH_3-N	NO_2^- + NO_3^- as N	Organic N	NH_3-N	NO_2^- + NO_3^- as N
1-31-80	O.E.C.D./Control				6.2	7.8	
2-05-80			19.3			0.84	
2-07-80				0			12.3
2-18-80		9.0	23.2		3.4	0	
2-26-80			22.4	0	7.0	0	20.2
2-28-80	Swisher/Control	10.6	26.6		3.9	0.56	
1-31-80	O.E.C.D./0.25% EBDC	6.2	17.6		14.0	19.6	
2-05-80						23.5	
2-07-80							0
2-18-80					1.1	23.5	0
2-26-80			23.5	0.6	1.1	25.2	0.3
2-26-80	Swisher/0.25% EBDC					5.6	6.72
2-28-80					3.9	0.84	
1-31-80	O.E.C.D./2.5% Whole Plant				3.9	27.2	
2-05-80						26.0	
2-07-80							0
2-18-80					2.0	28.8	
2-26-80			26.9			20.4	0
2-26-80	Swisher/2.5% Whole Plant					4.8	
2-28-80					3.6	0	8.40

Of further note is the fact that ETU removals were also greater in Swisher test units (Figures 15 and 16 and Table 19). Since thioureas are known inhibitors of nitrification (Thimann, 1963), the better ETU removal in the Swisher units may in part account for the better nitrification in those units. Maneb at 15 ppm levels has also been shown to inhibit nitrification in soils (Sanborn et al., 1977). A definitive confirmation of the effect of EBDC fungicides and/or ETU on nitrification should be of high priority in future studies. The Swisher units were operated at slightly higher temperatures than the O.E.C.D. units and higher temperatures favor the nitrification process. However, the O.E.C.D. control unit was operated at the lower temperature and showed nitrification was occurring at those temperatures.

While some ETU removal occurred in the units, detectable residues remained in the effluents (Table 19). Levels were in the tenths of mg/L. Since the National Academy of Science (NAS, 1977) has recommended that very strict criteria be applied for ETU in drinking water, the public health significance of discharge of these levels of ETU to surface waters should be assessed. However, activated carbon treatment should readily remove ETU (Little et al., 1980), and photolytic and other known degradation processes would tend to eliminate ETU from surface waters.

Compared to the wastewaters as received, the influents to the test units were considerably diluted, i.e., by a factor of 400 for the EBDC wastewater and by a factor of 40 for the whole plant wastewater. Therefore, the EC_{50} value for Daphnia would be expected to be greater than 100% for the 0.25% EBDC wastewater. As anticipated, no toxicity of either influent or effluent was noted. For the 2.5% whole plant wastewater the expected EC_{50} value for Daphnia would be greater than 40% based on dilution alone. As shown in Table 20, in the 2.5% whole plant wastewater influent, 100% of the Daphnia survived at a 32% dilution and 70% survived at 100% (undiluted). These data are consistent with the predicted EC_{50} value. In the effluents from the Swisher units fed 2.5% whole plant wastewater all the Daphnia survived while 70% survived in the undiluted (100%) O.E.C.D. effluent. While these data are sparse, they could be interpreted as indicating that biological treatment in the Swisher units relieved toxicity to some extent. However, at the concentrations tested, none of the wastewaters were remarkably toxic to Daphnia, either with or without treatment.

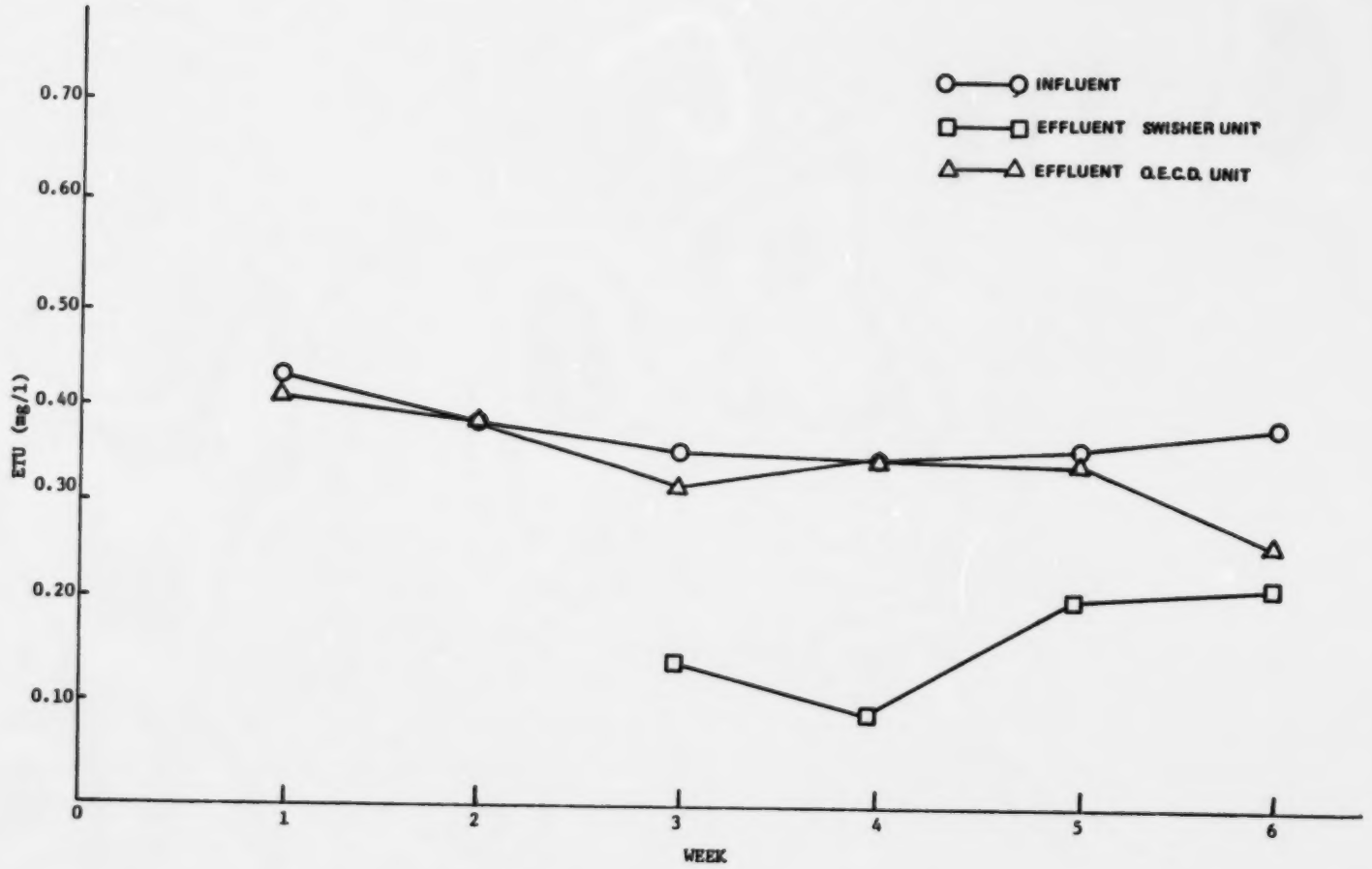


Figure 15. ETU Removal in Activated Sludge Units Treating 0.25% EBDC Wastewater.

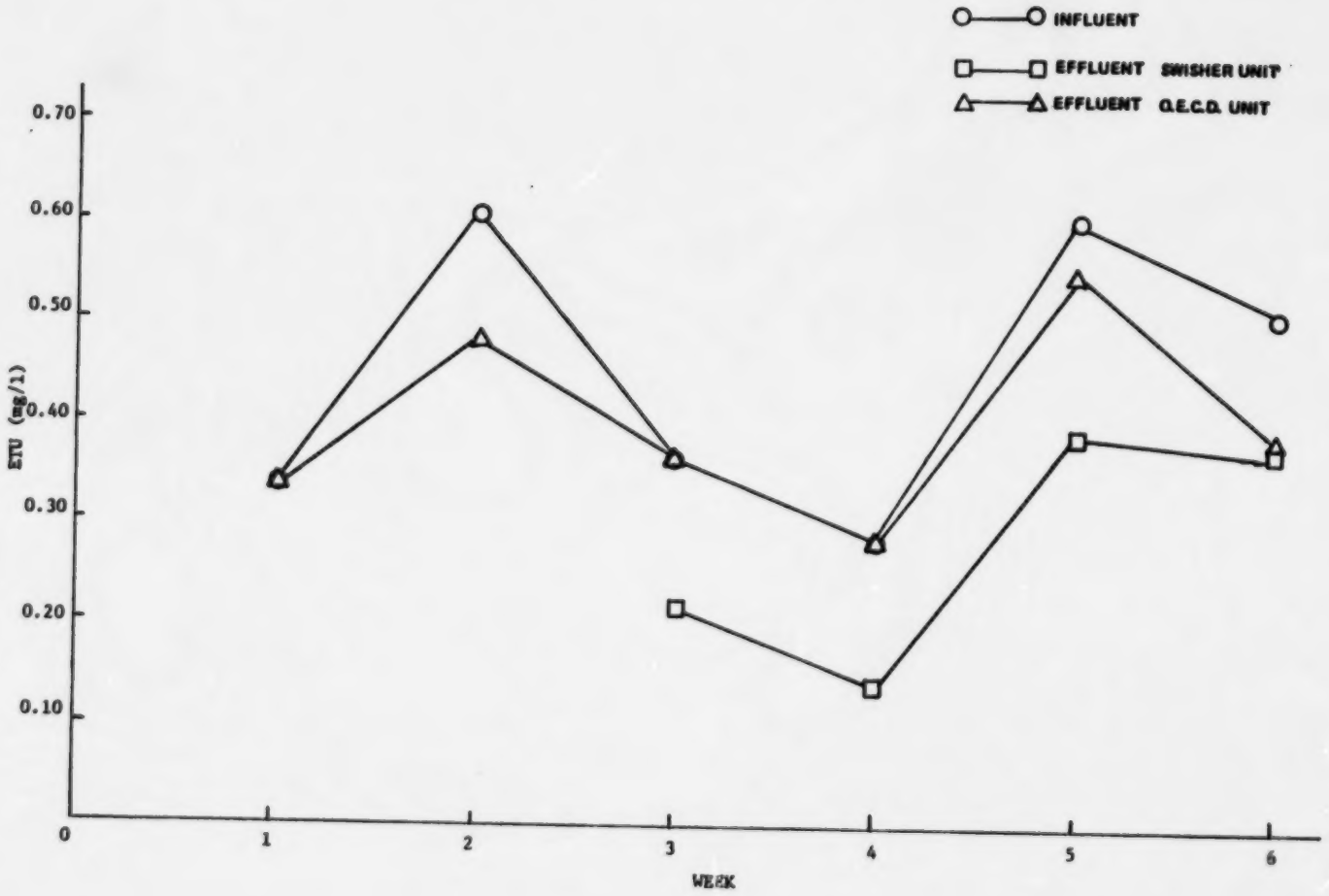


Figure 16. ETU Removal in Activated Sludge Units Treating 2.5% Whole Plant Wastewater.

Table 19. EBDC AND ETU CONCENTRATION IN INFLUENTS AND EFFLUENTS FROM SWISHER AND O.E.C.D. ACTIVATED SLUDGE UNITS

Type	Stream	ETU (mg/1) 2/7	2/13	2/18	2/28	3/6	3/10	EBDC and Related Compounds (mg/L) 3/16
0.25% EBDC	Influent	0.42	0.38	0.36	0.36	0.37	0.40	1.0
wastewater in municipal sewage	effluent swisher unit	-	-	0.14	0.10	0.22	0.24	0.75
	effluent O.E.C.D. unit	0.40	0.37	0.32	0.36	0.36	0.28	0.98
2.5% whole plant wastewater in municipal sewage	influent	0.34	0.61	0.37	0.29	0.62	0.52	ND
	effluent O.E.C.D. unit	-	-	0.22	0.14	0.39	0.37	ND
control (municipal sewage)	influent	ND	ND	ND	ND	ND	ND	ND
	effluent swisher unit	-	-	ND	ND	ND	ND	ND
	effluent O.E.C.D. unit	ND	ND	ND	ND	ND	ND	ND

ND = not detectable
<0.10 mg/1 ETU
<0.50 mg/1 EBDC and related compounds, Maneb used as a standard.

These results may be compared with the previous study (Little et al., 1980) which indicated that the untreated wastewater from the final synthesis step in maneb production had an LC_{50}-96 hr to fish of ~0.018%. A 1:10 dilution of primary sewage to which this wastewater was added at a 10% strength (v/v) killed all fish, but the effluents from units fed this mixture were much less toxic to fish.

Previous studies of the effect of maneb wastewater on algae showed that the 14-day EC_{50} was about 0.056% (v/v). The EBDC wastewater in the present study, more dilute than that of the previous study, had an EC_{50} between 1-10% (v/v). The whole plant wastewater EC_{50} levels were similar, i.e., around 1.0%.

Dilution in sewage reduced the inhibition to algae in both cases as would be anticipated (Table 21, Figures 17 and 18). The amount of variation in the algal assay results makes interpretation difficult (Table 21). However, the effluents from units treating the 0.25% EBDC wastewater were no more inhibitory to algae than were effluents from control units treating only municipal wastewater (Tables 21 and 22). The effluents from the units fed 2.5% whole plant wastewater appeared to be much more inhibitory. However, this effect was demonstrated only with the undiluted effluents. The conclusion might be drawn that, at the wastewater concentrations employed in these biological treatability tests, the acute algal toxicity of the effluents is low.

Table 20. DAPHNIA BIOASSAYS OF WASTEWATERS AND OF INFLUENTS AND EFFLUENTS OF ACTIVATED SLUDGE UNITS

Sample	Daphnia Surviving at 48 hr., %				
Dilution: % (v/v)	0.1	1	10	32	100
1. Whole plant wastewater	100	100	0		
2.5% whole plant wastewater					
- Influent to AS		100	100	100	70
- Effluent from Swisher			100	100	100
- Effluent from O.E.C.D.			100	100	
2. E.B.D.C. wastewater	100	80	0		
0.25% E.B.D.C. wastewater					
- Influent to AS		100	100	100	100
- Effluent from Swisher			100	100	100
- Effluent from O.E.C.D.			100	100	100
3. AS control units					
- Effluent from Swisher			100	100	100
- Effluent from O.E.C.D.			100	100	100

Table 21. ALGAL BIOASSAYS OF WASTEWATERS AND OF INFLUENTS AND EFFLUENTS OF ACTIVATED SLUDGE UNITS

Sample	Algal growth at 14 days, % of control					
Dilution: % (v/v)	0.01	0.1	1	10	56	100
1. Whole plant wastewater		86	52	4		
2.5% whole plant wastewater						
-Influent to AS		109	101	82		1
-Effluent from Swisher				72	42	7
-Effluent from O.E.C.D.				105	82	6
2. E.B.D.C. Wastewater	109	93	84	31		
0.25% E.B.D.C wastewater						
-Influent to AS		96	88	131		13
-Effluent from Swisher				141	59	46
-Effluent from O.E.C.D.				41	57	26
3. AS Control Units						
-Effluent from Swisher					57	25
-Effluent from O.E.C.D.					64	21

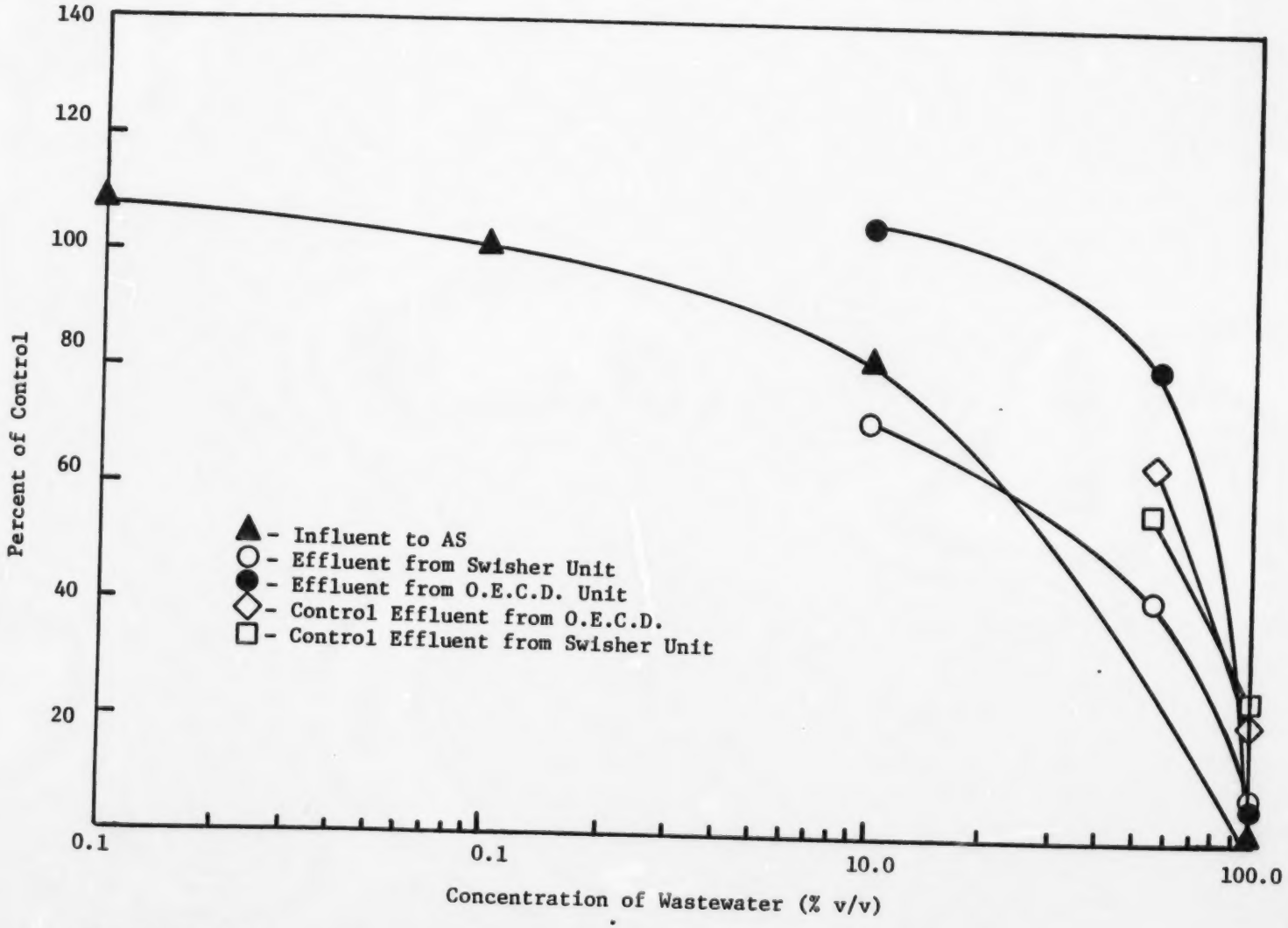


Figure 17. Algal toxicity of 2.5% whole plant wastewater before and after treatment as a percent of control.

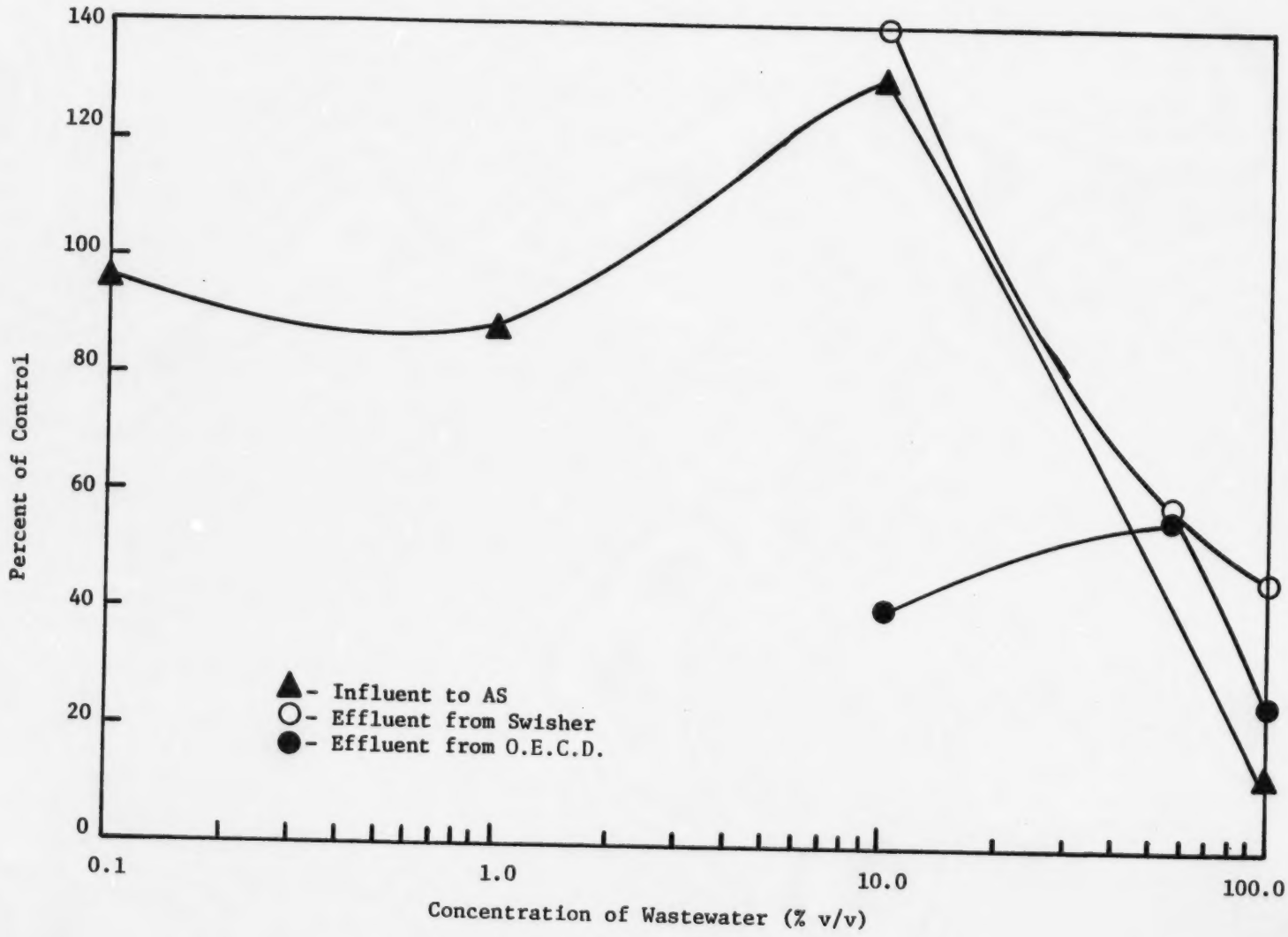


Figure 18. Algal toxicity of 0.25% EBDC wastewater before and after treatment as a percent of control.

Table 22. COMPARATIVE ALGAL TOXICITY OF EFFLUENTS FROM CONTROL AND TEST UNITS

Feed	Algal Growth at 14 days, % of Control		
	Dilution: % (v/v) 10	56	100
Municipal Wastewater Alone			
-Swisher	-	57 ± 68	25 ± 27
-O.E.C.D.	-	64 ± 32	21 ± 125
Municipal Wastewater + 2.5% whole plant			
-Swisher	72 ± 15	42 ± 18	7 ± 26
-O.E.C.D.	105 ± 49	82 ± 25	6 ± 60
Municipal Wastewater + 0.25% EBDC Wastewater			
-Swisher	141*	59 ± 28	46 ± 17
-O.E.C.D.	41 ± 17	57 ± 17	26 ± 59

*Based on one flask; Remainder are based on three flasks

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APPENDIX A

ANALYTICAL PROCEDURES FOR ROUTINE WASTEWATER CHARACTERIZATION

Routine wastewater analyses were conducted according to Standard Methods for the Examination of Water and Wastewater, 14th Edition, (APHA, AWWA, WPCF, 1975).

pH--

pH was determined electrometrically by Method 424.

Chloride--

Chloride was measured by the mercuric nitrate method (Method 408 B).

Alkalinity--

Alkalinity, as $CaCO_3$, was determined by Method 403.

Nitrogen Forms--

Total Kjeldahl nitrogen was determined after digestion, according to Method 421. Ammonia (NH_3-N) was determined by an acidimetric method as described in Sections 418 A and 418 D. Nitrite and nitrate nitrogen (NO_2-N, NO_3-N) were determined by the Devarda's alloy method (419 F).

COD--

Chemical oxygen demand (COD) was determined by Method 508.

Residues--

Suspended solids (SS) were determined by Method 208 D. Total solids (TS) were determined by Method 208 C. Total dissolved solids (TDS) were determined by Method 208 A.

Phosphate--

Total phosphate was determined by the Ascorbic Acid Method (425 F) after persulfate digestion according to Method 425 C.

APPENDIX B

PROCEDURES FOR CONDUCTING ACTIVATED SLUDGE TREATABILITY STUDIES

For biological treatability studies the bench scale, complete-mix, continuous-feed, activated sludge units designed by Swisher (1970) and the Organization of Economic Cooperation and Development (O.E.C.D.) (Bundegestzblatt, 1977) (Figures B-1 and B-2 respectively) were employed. These units are made entirely of glass, avoiding the possibility of contamination by organics leaching from the container. Continuous feed to the units was supplied through Teflon tubing by a peristaltic pump to give the nominal retention time of 8 hours.

Routine determinations were made of dissolved oxygen, pH, mixed liquor volatile suspended solids in the aerator, COD, and pesticides in the influent and effluent. Dissolved oxygen was determined with an oxygen probe (Yellow Springs Instrument Company).

Solids were wasted from the AS units by either dipping or aspirating a portion of the liquor from the aeration chamber. The target total solids was 3-4 g/L.

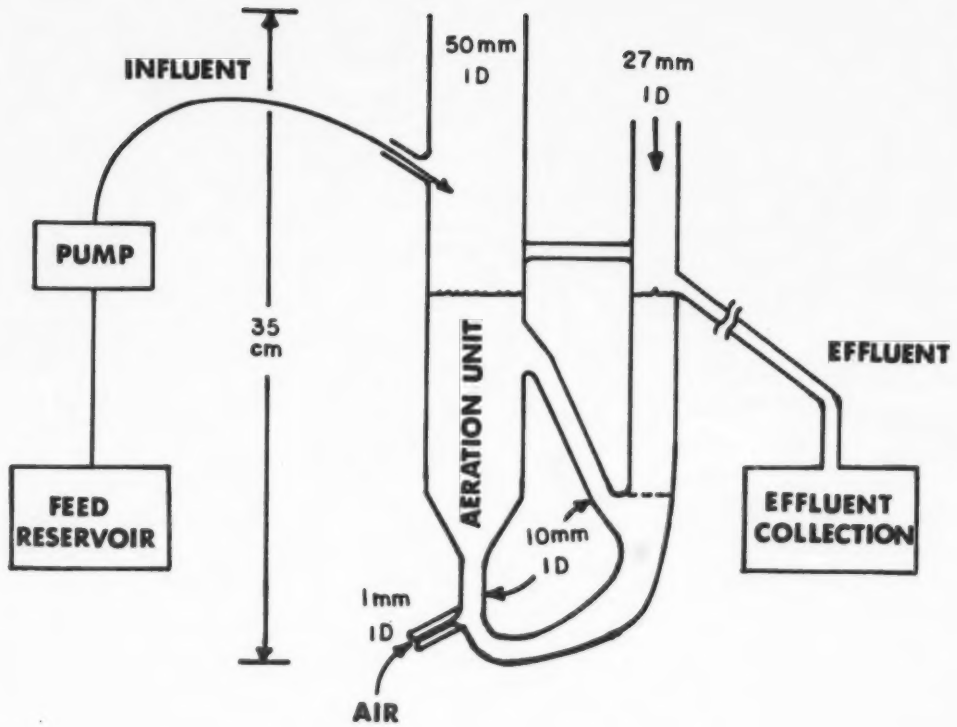

Figure B-1. Diagram of Swisher Activated Sludge Pilot Unit.

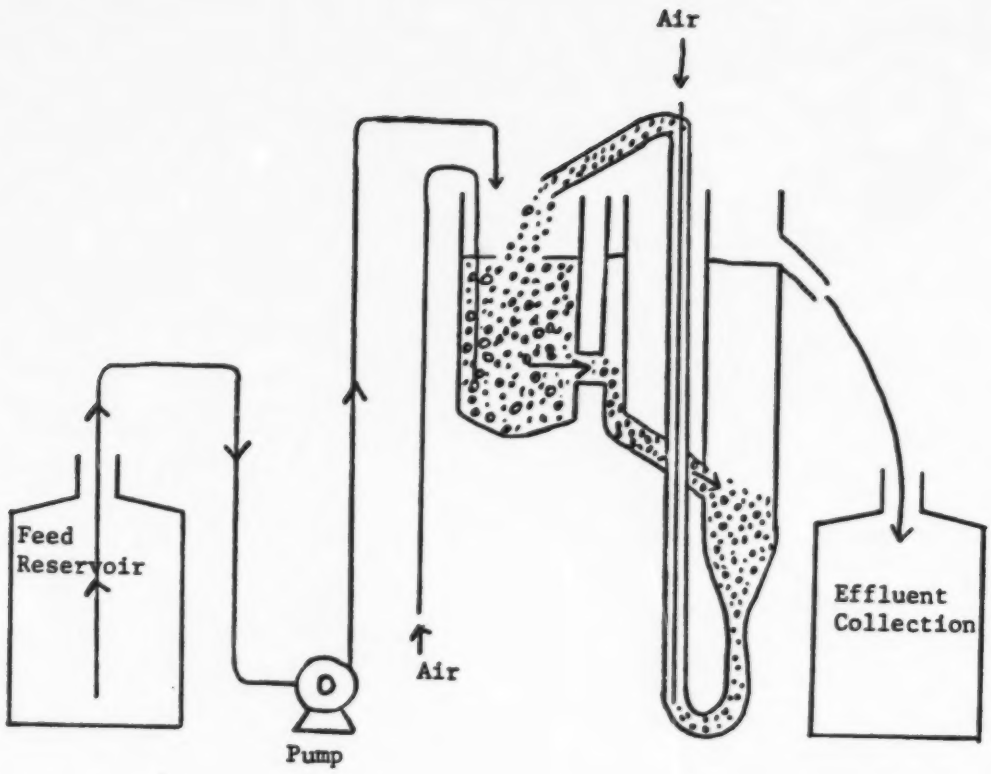

Figure B-2. Diagram of O.E.C.D. Activated Sludge Pilot Unit.

APPENDIX C

PROCEDURES FOR ALGAL ASSAY TESTS

Algal bioassays were conducted according to the freshwater algal assay procedure described in the report The Selenastrum Capricornutum Printz Algal Assay Bottle Test (Miller, Greene, and Shiroyama, 1978). The test alga was Selenastrum capricornutum Printz, obtained from the National Eutrophication Research Program, EPA, Corvallis, Oregon. This test was designed to measure algal response to changes in nutrient concentrations and to determine toxicity or inhibition.

Wastewaters to be tested were filter sterilized through a sterile prewashed membrane filter (Millipore Filter, 0.45 μm pore size). Serial dilutions were then made in sterile algal media to give the appropriate final concentration. Sufficient inoculum was added to produce an initial cell concentrations of 10^4 cells/mL.

In each set of experiments, algal growth in the presence of a series of concentrations of the wastewater added to the nutrient medium was compared to algal growth in the nutrient medium alone. Growth was determined by direct counts of the algae during the 10-14 day incubation period. Effect of the wastewater on algal growth was determined in terms of the effect on the cell yield. Direct cell counts were performed by an automated procedure utilizing a Fisher Scientific Model FO 16 particle counter.

The tests were conducted in water bath shakers at 24 $\pm$ 2°C and at approximately 80 oscillations per minute with constant white fluorescent lighting at 4300 lux. Test containers were 250 mL Erlenmeyer Pyrex flasks containing 100 mL of test medium and covered with an inverted Pyrex beaker.

The method of expressing inhibitory or stimulatory effects was that recommended by Miller et al. (1978), i.e., as the percent growth inhibition (I) or stimulation (S), as compared to growth in a control culture without the test materials. These authors suggest that in general practice, the results

be based on the growth at 14 days, i.e., as % I_{14} or % S_{14} at a given concentration of the effluent being tested. Typical cell counts for controls on day 14 of a test were between 5 and 6 million cells per mL.

Cell counts were performed using a Royco Model 920A Cell Counter (Royco Instruments, Inc., Menlo Park, CA). Each dilution is run in triplicate and each replicate counted three times for a total of nine measurments per dilution.

Decreased growth, compared to the control, is evidence of an inhibitory effect. The manner in which the test is conducted does not allow determination of whether this inhibition is temporary (algistatic) or permanent (algicidal). Such a determination would require further testing by subculturing into fresh medium free of the test material.

APPENDIX D

PROCEDURE FOR DAPHNIA BIOASSAY TESTS

The 48 hour, static Daphnia bioassay was adapted from the procedures outlined by Duke et al (1977). Daphnia magna was selected as the test species. An original population of Daphnia magna was obtained from W. T. Waller of the University of Texas at Dallas. The Daphnia used in these bioassays were reared at Research Triangle Institute under conditions which promote parthenogenetic reproduction. In the RTI procedure, each member of the breeding stock is reared in isolation. When reproduction occurs young are removed within 24 hours. Ages of the breeding stocks are staggered and each daphnia breeding stock is replaced after 56 days. All Daphnia in the present breeding population are the offspring of a single Daphnid.

Daphnia are reared in deionized water which is reconstituted to standard hard water specifications by addition of 192 mg $NaHCO_3$, 120 mg each of $CaSO_4 \cdot 2H_2O$ and $MgSO_4$ and 8 mg of KCl per liter of water. Daphnia are fed daily portions of Selenastrum capricornutum culture except during testing periods.

Range finding bioassays may be conducted with a mixed population of Daphnia. All narrow range, definitive bioassays are conducted with first instar young.

Tests are conducted in 250 ml beakers to which 100 ml of combined test solution and reconstituted hard water have been added as noted in the test. Ten Daphnia are randomly assigned to each condition. Survival of the Daphnia at 24 and 48 hours is judged by careful examination for mobility. Survival of Daphnia under control conditions must be 90% or better for a bioassay to be considered valid. Occassionally the controls exhibited a phenomenon we referred to as "floating". The Daphnid becomes trapped at the surface of the water and is held there by the surface tension of the water. This phenomenon has never affected the young of the stock breeding population. This "floating" phenomenon is also quite intermittant and occurs only at widely scattered

intervals in our acute testing program. When it does occur it seems only to affect the control Daphnia and the Daphnia in the lowest concentrations of test solution while higher nonlethal concentrations are unaffected. When it does occur, most or all Daphnia in these low contaminant conditions are affected. The problem is not unique to our laboratory. W. T. Waller of the University of Texas at Dallas reports using screens to keep daphnia below the surface during long term assays to avoid this phenomenon. Where this phenomenon occurs during acute testing the bioassay is repeated.

APPENDIX E

ANALYTICAL METHODS FOR SPECIFIC SUBSTANCES

PROCEDURE FOR ANALYSIS OF ETU IN WASTEWATER

Gas Chromatography Method

Adapted from Haines and Adler, "G. C. Determination of ETU residues", J.A.O.A.C. 56:333-337, 1973.

Clean-up Procedure--

100 mL of wastewater was diluted to 300 mL with methanol. A 100 mL aliquot was run through a 2.5 cm diameter clean-up column consisting of 3 layers:

Top: 2.5 g 60/100 Florisil with 2% H_2O added

Middle: 10.0 g alumina activity II-III

Bottom: 2.5 g Celite 545

The column was washed with 50 mL methanol and eluate collected in a round-bottom flask. Five mL dimethylformamide (DMF) was added to the 150 mL of column eluate, and the solution evaporated on a rotary evaporator at 55°C to a volume of 2-3 mL.

Derivatization--

The concentrate was diluted to 6.0 mL with DMF in a test tube; 0.3 mL bromobutane, ~ 0.05 g sodium borohydride and 1.0 mL 20% NaOH were added. The tube was shaken, then allowed to sit for 5 min to react. 4.0 mL of the upper phase was removed to a 50 mL round bottom flask and 1 drop concentrated HCl added. The solution was concentrated to dryness on a rotary evaporator at 90-100°C. The residual solids were dissolved in 0.5 mL distilled H_2O, 0.5 mL 20% NaOH, and 0.5 mL cyclohexane/ethyl acetate (4:1) and shaken for 1 min. The upper phase was removed to a small vial for analysis.

Preparation of ETU-S-derivative--

Prepared according to procedure in Haines and Adler, J.A.O.A.C. 56:333-337, 1973. Confirmed by melting point and UV spectrum.

Gas Chromatography--

Gas chromatography was performed on a Varian 3700 gas chromatograph equipped with a flame photometric detector. A 10% Carbowax 1500 + 2% KOH on Chromosorb WHP 80/100 column (180 cm x 2 mm id) was used. The carrier gas flow was set at 30 mL/min. Column temperature was 170°, injector temperature 200°, detector temperature 200°. Injection volume was 5 µL. Minimum detectable quantity was 25 ng with a standard deviation of 3.7% at 5 mg/L. Recoveries of cleaned-up and derivatized samples were very poor (~ 25%). Derivatized samples of standard solutions of ETU gave ~ 95% recoveries, indicating the problem is not in the derivatization step. ETU was present in column eluate, as determined by thin layer chromatography hence it is not being lost in the column. Given these problems in applying the method to this matrix, an alternate method given below was used.

High Performance Liquid Chromatography (HPLC)

A modular liquid chromatographic system was used for the analyses. The basic system consisted of the following components: 2 M6000 pumps with a M660 solvent programmer and a U6K injector (Waters Associates), a Model SF-770 Variable Wavelength Ultraviolet Detector (Schoeffel Instruments), and a Bioanalytical Systems, Inc. electrochemical detector (U-2A controller with TL-8A cell). A reverse phase column packed with Lichrosorb RP-18 (E. M. Merck) was employed.

A mobile phase of 4% Methanol-H_2O buffered to pH 5.5 with 0.05M phosphate was pumped through the column at 1.0 mL/min. The ultraviolet detector was used at a wavelength of 240 nm, while the electrochemical detector was used with a potential of 0.9 volts vs Ag/AgCl reference electrode and attenuation of 5 nanoamps/volt. Sample injection size was 25 µL. All samples were filtered through a 5.0 µ Millipore LS filter prior to direct injection on the HPLC system.

Minimum detectable quantity was 2.5 ng with a standard deviation of 3.3% at 0.1 mg/L ETU.

ETHYLENE BISDITHIOCARBAMATE SALTS

Gas Chromatography/Electron Capture Detection Method

The procedure for maneb determinations was adapated from McLeod and McCully (1969) and involves reduction of maneb to produce carbon disulfide (CS_2) using stannous ion, followed by measurement of CS_2 by GC/ECD. This method does not provide information on speciation of the dithiocarbamate breakdown products and is sensitive to the disulfide and monosulfide cyclic thiocarbamates as well as the metal complex. The method is insensitive to ethylenethiourea, a stable product of maneb oxidation. Maneb was used as the standard and results are reported as if all CS_2 were due to maneb.

The maneb reference standard (98% pure) was obtained from the Quality Assurance Section, Environmental Toxicology Division of EPA, Research Triangle Park, N.C. The CS_2 standard (certified ACS) was obtained from Fisher Scientific Co. The stannous chloride reagent was prepared fresh daily from the ACS grade chemical (Fisher Scientific Co.) by dissolving 1.5 g in 100 ml of 6 M HCl. Versene solution was prepared by diluting 93 g of ACS grade disodium-ethylenediaminetetraacetate (Fisher Chemical Co.) to 1 liter with deionized water. Standards were prepared by weighing 400 mg of maneb into a 100 mL volumetric flask and diluting to volume with the Versene solution. This standard was in turn diluted with Versene solution to produce the calibration standards. Standards and samples were processed by placing 1 mL into a 60 mL narrow mouth amber bottle along with 9 mL of the stannous chloride reagent. The bottles were then closed with septum caps and shaken in a 65°C water bath for 30 minutes. For analysis, 0.4 mL of headspace was withdrawn and analyzed by GC/ECD.

Gas chromatography was performed with a Varian 1400 gas chromatograph equipped with a tritium scandide detector. The glass column (170 x 0.2 cm id) was packed with 0.2% Carbowax 1500 on 60/80 mesh Carbopack C preceded by a 2.5 cm long plug of anhydrous sodium sulfate. The oven and injection port were held at ambient temperature ($\sim$ 28°C) and the detector was held at 280°C. Nitrogen was used as carrier gas at a flow of 15 mL/min and as makeup gas at a flow of 5 mL/min.

A calibration curve was generated showing a linear response over the range of 0.5-40 mg/L of maneb with a correlation coefficient of 0.9929. Results are shown in Table E-1.

Table E-1. DETERMINATION OF ETHYLENE BISDITHIOCARBAMATE SALTS AND RELATED COMPOUNDS

Sample	Concentration (mg/L)[a]
EBDC Wastewater	41.6 ± 5.8
EBDC Influent[b]	0.997 ± 0.044
EBDC Effluent - O.E.C.D. unit	0.980 ± 0.031
EBDC Effluent - Swisher unit	0.754 ± 0.049
Whole Plant Wastewater	16.0 ± 0.42
Whole Plant Influent[c]	[d]
Whole Plant Effluent - O.E.C.D. unit	N.D.[e]
Whole Plant Effluent - Swisher unit	[d]
Control Influent	[d]
Control Effluent - O.E.C.D. unit	[d]
Control Effluent - Swisher unit	[d]

[a] Average ± absolute deviation determined by duplicate analyses.

[b] A 0.25% dilution of the EBDC wastewater.

[c] A 2.5% dilution of the Whole plant wastewater.

[d] Less than 0.5 mg/L, the detection limit.

[e] No data.

PROCEDURE FOR ANALYSIS OF MANGANESE AND ZINC

Wastewater samples were analyzed for manganese and zinc on a Perkin-Elmer model 403 Atomic absorption spectrometer equipped with graphite furnace and deuterium arc background correction system.

Samples were filtered before analysis. No further workup was done. Analysis was performed using aqueous standards. Deionized water was used as diluent when necessary. Two samples were run for manganese and eight for zinc.

Operating Conditions

Zn--

Wavelength 308 nm
Slit Setting = 4
Nitrogen purge gas with automatic gas interrupt
20 µL injections (50 µL injections were used for lowest level samples)
Recorder Response = 2
Recorder Range = 5 mv
Scale expansion with concentration control = 300
Furnace conditions were:
Dry 200°C, 20 seconds
Char 500°C, 20 seconds
Atomize 2000°C, 8 seconds

Mn--

Wavelength 279 nm
Slit Setting = 4
Nitrogen purge gas with manual gas interrupt
20 µL injections
Recorder Response = 2
Recorder Range = 5 mv
Absorbance mode
Furnace conditions were:
Dry 200°C, 20 seconds
Char 1000°C, 20 seconds
Atomize 2500°C, 8 seconds

A linear least squares calibration technique was used.